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CALIFORNIA ENERGY COMMISSION

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End-Use Energy Efficiency

INTEGRATED AGRICULTURAL TECHNOLOGIES DEMONSTRATIONS

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Preface

The Public Interest Energy Research (PIER) Program supports public interest energy research and development that will help improve the quality of life in California by bringing environmentally safe, affordable, and reliable energy services and products to the marketplace.

The PIER Program, managed by the California Energy Commission (the Commission, Energy Commission), annually awards up to $62 million through the Year 2001 to conduct the most promising public interest energy research by partnering with Research, Development, and Demonstration (RD&D) organizations, including individuals, businesses, utilities, and public or private research institutions.

PIER funding efforts are focused on the following six RD&D program areas:

- Buildings End-Use Energy Efficiency
- Industrial/Agricultural/Water End-Use Energy Efficiency
- Renewable Energy
- Environmentally-Preferred Advanced Generation
- Energy-Related Environmental Research
- Strategic Energy Research.

In 1998, the Commission awarded approximately $17 million to 39 separate transition RD&D projects covering the five PIER subject areas. These projects were selected to preserve the benefits of the most promising ongoing public interest RD&D efforts conducted by investor-owned utilities prior to the onset of electricity restructuring.

Edison Technology Solutions (ETS) is an unregulated subsidiary of Edison International and an affiliate of Southern California Edison Company (SCE). As a result of a corporate restructuring, ETS ceased active operations on September 30, 1999. ETS' remaining rights and obligations were subsequently transferred to SCE.

What follows is the final report for the Integrated Agricultural Technologies Demonstrations project**,** 1 of 10 projects conducted by ETS. This project contributes to the Industrial/Agricultural/Water End-Use Energy Efficiency program.

For more information on the PIER Program, please visit the Commission's Web site at: http://www.energy.ca.gov/research/index.html or contact the Commission's Publications Unit at 916-654-5200.

Executive Summary

California leads the nation in agricultural production, generating $25 billion in revenue in 1998. The State produces more than 200 crops for both export and domestic consumption. One of every three California jobs is directly or indirectly related to agriculture production, storage, food processing, or distribution. Food production alone accounts for six percent of the State's employment.

Major challenges currently face California's agricultural community. Increasingly stringent environmental and regulatory controls mandate changes in the use and disposal of agricultural chemicals; require the more aggressive management of farm wastes; and impose new responsibilities for water use and protection of its quality. As a consequence, farmers are actively seeking alternative methods and practices for the new millennium.

Methyl bromide is one of the most widely used chemicals by the agricultural community. This chemical has been used for years as an effective fumigant and fungicide in the cultivation and storage of many of California's crops. The chemical will be banned from use in the United States by the Year 2003.

In addition, many of the disinfectants and fungicides commonly used by the agricultural industry, have been labeled as known or potential human carcinogens by the U.S. Environmental Protection Agency (EPA) and more restrictive control of their usage can be anticipated in the near future. Human health concerns encourage the search for less hazardous disinfection and anti-fungicidal alternatives.

Modern livestock farming practices have become more intense and led to the development of factory farms characterized by large animal populations. The waste from these animals is both odiferous and rich in nutrients, particularly nitrogen and phosphorus. Management of these wastes has become more increasingly problematic. Traditional methods of waste management have led to regulatory actions in response to air quality and water quality concerns.

Competitive demands on the state's limited fresh water supply have also emerged. Historically, agriculture has been the largest user of water to support irrigation. Farmers are increasingly asked to modify historic irrigation strategies using large quantities of water in wasteful flood and spray irrigation technology practices.

This program was conceptualized to analyze and demonstrate a number of energy efficient and environmentally friendly technologies designed to address these issue. Findings will be used to help California farmers enhance competitiveness; improve productivity; and reduce the use of toxic chemicals, energy consumption, and water usage.

Specifically, this program sought to demonstrate technologies in six different areas. These projects and their outcome and recommendations are discussed in the following.

<u>Ozone in Soil Fumigation:</u>

Objective

- The effectiveness of ozone as a preplant soil fumigant to destroy a variety of soil-borne microorganisms (Section 2.1).

Outcomes

- Ozone treatment demonstrated substantial improvements and crop yield or plant vigor compared to untreated controls, in all crops tested except peaches
- Soil treatment with ozone decreased soil pathogens and increased nutrient availability.

Recommendation

- Additional research is needed to optimize the use of ozone to maximize yields on various crops, in a variety of soil types and climatic conditions.

Ozone as an Aqueous Disinfectant

Objective

- The effectiveness of ozone as a disinfectant and fungicide in aqueous solutions used for fruit storage and packaging operations and ozone treatment for discharge water quality purposes (Section 2.2).

Outcomes

- From 95 to 100 percent of all eight fungi tested were killed in 2 minutes of contact time with the ozone treatment. None survived 3 minutes of contact
- Ozone effectiveness in water to kill spores of *Penicillium digitatum* was not influenced by pH and could not be predicted by a dose and time relationship
- High doses of ozone were required to kill pathogens on fruit surfaces than those needed to kill spores in water
- Ozone significantly reduced gray mold incidence on table grapes, but its efficacy was irregular
- Ozone was inferior to sodium hypochlorite, sodium bicarbonate and ethanol as a fungicide
- The control of pathogens inoculated into wounds in citrus fruit failed even after prolonged treatment with very high ozone concentrations in water
- Table grapes, citrus fruit, and strawberries were not visibly injured by the ozone treatments evaluated
- In strawberry wash water, ozonation for 3 hours greatly reduced microbe populations, moderately reduced biochemical oxygen demand, chemical oxygen demand, and suspended solids, did not reduce total organic carbon, and increased total dissolved solids.

Recommendations:

- This project was the first phase in a three-phase program. As such, results are preliminary and more research is required.
- Ozone could have a role in reducing fungicide residues in discharge water. More research to assess the benefits on ozone in water should be conducted.

Ozone as a Gas Fumigant:

Objective

- The effectiveness of ozone as a post-harvest fumigant to control insect infestation of fresh and dried fruits and vegetables (Section 2.3).

Outcomes

- Four to 6 hours of exposure to ozone concentrations of 300 to 500 parts per million were needed to kill Indianmeal moth larvae and diapausing codling moths
- Chambers designed for ozone fumigation will need to be made of materials that can withstand the corrosive action of continuous exposure to high concentrations of ozone.

Recommendation:

- This project is the first phase of a three-phase program. Results of this first phase are preliminary and more research is required.

Alternative Fruit Storage Methods:

Objectives

- Demonstrate the viability and practicality of a low-cost, temperature-controlled storage facility to inhibit insect infestation of stored fruit (Section 2.4.2)
- Test the effectiveness of plastic film bin liners to control insect control in stored prunes (2.4.3)
- Develop baseline data on Indianmeal moth populations near drying and storage facilities (Section 2.4.4).

Outcomes:

- The controlled ventilation/evaporative cooling system was too expensive to install considering it would provide safe storage temperatures for 7 months of the year
- Prune quality was determined to be excellent after 1 year in storage in plastic bags
- Moisture content of the prunes did not change during storage and the fruit was exceptionally free of sugaring and mold compared to fruit stored conventionally
- It appears that bins with liners must be stored in an environment that prevents significant diurnal temperature fluctuations
- Baseline data collected indicated that Indianmeal moth is the species of most concern within prune storage
- The liner storage system will work well only is fruit is virtually free of infestation before being placed in the liners.

Recommendations:

- No further research is required in the use of a controlled ventilation/evaporative cooling unit.
- Additional research with improved bin liner bags is needed to determine the effectiveness of this approach.

Dairy Wastewater Management:

Objective

- The functionality of an innovative biological treatment device to manage the disposal of liquefied animal wastes (Section 2.5).

Outcomes

- The sequencing batch reactor was found to be an effective biological reactor for treating dairy wastewater
- A two-stage sequencing batch reactor system is recommended over a single-stage system if nutrification is desired
- The two-stage system was capable of achieving near-complete conversion of ammonia to nitrite and nitrate in the dairy wastewater.

Recommendation

- This is the first phase of a three-phase program. A full-scale demonstration is recommended to complete project objectives.

Irrigation Scheduling:

Objective

- To investigate new and advanced techniques for improving irrigation efficiency for fruit and nut orchard crops (Section 2.6).

Outcomes

- Linear variable displacement transducers can be used as a surrogate for stem water potential. This gives farmers a method to electronically monitor plant water status that identifies immediately when a plant enters stress due to lack of water.
- Measuring shaded leaf water potential can be used as a surrogate for stem water potential.

Recommendation:

- Only 3 months of data have been collected to date. It is recommended that field trials be continued to their scheduled conclusion at which time more meaningful research results should be available.

While these innovative applications are not ready for immediate commercial deployment, a majority of the research had promising results. It is likely that this technology, in the very near future, will have the potential to resolve most of the environmental, water, and energy issues threatening the success and competitiveness of this very import segment of California's domestic economic product.

Additional research and demonstration funding will be required to accelerate the commercial deployment of these technologies and make their potential economic benefit a reality for California's farmers.

Abstract

California leads the nation in agricultural production, generating $25 billion in revenue in 1998. Major challenges currently face California's agricultural community including the increasingly stringent environmental and regulatory controls. This program was conceptualized to analyze and demonstrate energy efficient and environmentally friendly technologies to address these challenges. Program projects investigated and demonstrated advanced agricultural technologies including: the use of ozone as a soil fumigant, as an aqueous disinfectant, and as a gas fumigant for insect control; the effectiveness of a controlled ventilation and evaporative cooling system and plastic bin lines for prune storage, effective separation of manure solids and ammonia removal from residual liquid cattle wastes, and the use of new technology for improved irrigation scheduling.

The use of ozone as a soil fumigant demonstrated broad and impressive beneficial effects. Ozone showed promise when used as an aqueous disinfectant to minimize chemical or microbial contamination of agricultural processes water. Ozone's effectiveness as an insect fumigant requires a relatively long exposure period and the corrosive nature of the chemical limits the selection of exposure chamber materials. The controlled ventilation/evaporative cooling system for dried prunes was not an economically attractive option when compared to other equally effective storage alternatives. The use of plastic bin liners for post-harvest storage showed great promise. A sequencing batch reactor was shown to be an effective biological treatment system for dairy manure management. Preliminary observations from the scheduled irrigation demonstration task point to the usefulness of developing an easily collected proxy indicator that can be used to electronically determine plant and soil conditions as inputs to irrigation schedule development.

1.0 Introduction

California leads the nation in agricultural production with annual revenues in excess of $25 billion. California farmers account for more than 10 percent of national farm income. The State produces more than 200 cash crops for export and for domestic consumption.

One in three jobs in California are either directly or indirectly related to agriculture production and food processing. Food production provides 6 percent of the State's jobs. Farmers in the Southern California Edison service area provide in excess of $244 million annually of electric revenue; more than 40 percent of the energy use in the Central Valley is in Edison service territory.

Notwithstanding this success, the future for farming in California is clouded. Increasingly stringent environmental regulations preclude the use of some chemicals that have long provided reliable control of crop pests and protected the appearance and freshness of market crops. In addition, water and air quality concerns require farmers to more proactively manage wastes produced by farm animals and growing competition with urban areas mandate the implementation of advanced irrigation practices.

1.1 Program Purpose

This program addressed the following challenges to California farmers:

- Soil fumigation practices
- Insect and fungi control of post-harvested fruits and vegetables
- Manure management
- Irrigation.

1.2 Program Objectives

Specifically, this program sought to demonstrate:

- The effectiveness of ozone as a preplant soil fumigant to destroy a variety of soil-borne microorganisms (Section 2.1)
- The effectiveness of ozone as a disinfectant and fungicide in aqueous solutions used for fruit storage and packaging operations and ozone treatment for discharge water quality purposes (Section 2.2)
- The effectiveness of ozone as a post-harvest fumigant to control insect infestation of fresh and dried fruits and vegetables (Section 2.3)
- The practicality of using low temperature control and ventilation strategies and/or plastic bin liners as substitutes for methyl bromide in the insect control of stored prunes (Section 2.4)
- The functionality of an innovative biological treatment device to manage the disposal of liquefied animal wastes (Section 2.5)
- To investigate new and advanced techniques for improving irrigation efficiency for fruit and nut orchard crops (Section 2.6).

1.3 Purpose and Organization of this Report

The purpose of this report is to provide detailed findings of the Integrated Agriculture program and provide recommendations for the continuation of this work.

This program was organized into six distinct research projects:

- Ozone in Soil Fumigation (Section 2.1)
- Ozone as an Aqueous Disinfectant (Section 2.2)
- Ozone as a Gas Fumigant (Section 2.3)
- Alternative Fruit Storage Methods (Section 2.4)
- Dairy Wastewater Management (Section 2.5)
- Irrigation Scheduling (Section 2.6).

2.0 Technical Discussion

The following narrative presents each of the research projects that comprise this program, in detail.

2.1 Ozone as a Soil Fumigant

Soil fumigation is widely practiced throughout the United States to destroy a variety of soil-borne microorganisms prior to planting. Many commercial crops are vulnerable to attack by bacteria, fungi, and nematodes, which collectively cause billions of dollars each year in crop losses.

The most popular fumigant, methyl bromide, is extremely effective as a soil-fumigating agent and is the most widely used fumigating agent with the broadest biocidal spectrum currently available. As a result, use of the chemical has grown exponentially over the past 20 years and now amounts to about 55 million pounds (lbs.) per year in the United States for soil preplant fumigation. Because of its deleterious long-term effects on the ozone layer and human health and safety concerns, however, methyl bromide use is being eliminated in the United States. Under the Montreal Protocol, to which the United States is a signatory, a gradual phase-out of methyl bromide beginning in 1999 is to be complete by the Year 2005.

Currently, there is no perfect substitute that will be available when methyl bromide is ultimately banned. Most alternative chemical treatment have substantial human health and safety risks or environmental persistence or toxicity problems associated with their use. For instance, Telone (a mixture of Dichloropropene and Chloropicrin) and Vapam (containing metam sodium) are both on the Proposition 65 list in California. This listing identifies Telone and Vapam as hazardous materials, restricting and controlling their use. Telone's use throughout California is limited because of its air pollution potential and it is expected that similar restrictions will be put on Vapam in the near future.

Other fumigants either have strong odors which preclude their application near residential areas or display severe phytotoxicity (plant poisoning) characteristics if planting follows application too quickly.

Other alternatives have not demonstrated efficacy on a broad basis and are encumbered with obstacles to broad-based application. These alternatives include steam sterilization (large water requirements), plastic mulch solarization (time duration required and disposal of contaminated used plastic), organic fortification such as manure or cole crop residues (limited large-scale availability and limited efficacy), and cultural practices such as cropping, cover crops, and field sanitization (limited efficacy).

Because ozone is unstable and rapidly breaks down, it cannot be stored and transported and must be produced onsite and used immediately. Commercially, ozone is produced from oxygen in ambient air through an electrical discharge process with relatively simple pieces of equipment known as ozone generators. Ozone is produced by passing oxygen or air through this electrical field, causing a certain percentage of the oxygen molecules to dissociate and then recombine as ozone.

In contrast to methyl bromide and other alternative fumigants, ozonation is easily applied and causes no adverse air quality effects if released to the atmosphere.

Ozone has a half-life of 12 hours or less in the atmosphere and degrades simple diatomic oxygen as its decomposition product. The technology can be easily incorporated into existing application practices, and requires no onsite transportation, storage, handling, or discharge of toxic chemicals. The following narrative summarizes the potential environmental and human health and safety benefits of using ozone as a soil fumigant:

- **Onsite Manufacture – No Transportation, Storage, or Discharge of Hazardous or Toxic Chemicals**. Ozone is manufactured on site and at low pressures. It is not stored and is immediately consumed in the soil treatment process. A widespread sudden release of ozone into the atmosphere that would be harmful to humans cannot occur as it can with compressed methyl bromide or other persistent, toxic gases or chemicals.
- **No Environmentally Persistent Chemicals Left in Soil**. Ozone has a very short half-life of minutes or less in soil with simple diatomic oxygen as its decomposition or reaction product. Use of ozone in soil treatment does not result in the buildup of any environmentally persistent and toxic compounds.
- **No Reentry, Permitting, or Use Restrictions**. Ozone is regulated by the U.S. Environmental Protection Agency (EPA) as a "biocidal device" and is thus exempt from further registration requirements by the EPA for state regulatory agencies. The California Department of Pesticide Regulation has confirmed this interpretation in writing. In addition to the associated lack of permitting requirements, the short half-life of ozone allows virtual immediate reentry after application without any risk of adverse exposure to workers.
- **Minimum Human Acute and Chronic Toxicity – No Human Carcinogenicity or Teratogenicity**. Except in extremely rare cases of extended, severe overexposure to high concentration of ozone (several hours at greater than 2 to 3 parts per million), the physical symptoms of ozone exposure are transitory in nature. Indeed, ozone has been used commercially in water treatment for over 100 years in tens of thousands of installations without a single recorded fatality.
- **No Broad Spectrum Environmental Toxicity**. In stark contrast to aqueous phase applications of ozone, ozone treatment of soil shows only a weak or nonexistent biocidal effect on many soil organisms and pathogens. Ozone is not a broad-spectrum biocide and it does not destroy or impair the soil microflora. To the contrary, in soil treatment ozone actually stimulates the growth of certain beneficial soil-borne microorganisms by increasing naturally occurring soil-bound nutrients—making them available for and resulting in increased plant growth. Ozone is a natural product that naturally interacts with soils and their constituents. Ozone's use as a preplant treatment merely mimics and enhances the same natural oxidative soil processes that are already occurring in the soil.
- Although ozone is considered an airborne pollutant when formed in the atmosphere by photosynthic reactions of nitrogen compounds, the use of ozone in aqueous applications is actually exempted from regulation by many regional air pollution control districts. For instance, the South Coast Air Quality Management District, the country's largest air pollution control district covering most of Southern California, has exempted ozone from all permitting requirements for aqueous applications.

2.1.1 Project Objective

This project sought to demonstrate the effectiveness of ozone as a preplant soil fumigant to destroy a variety of soil-borne microorganisms. The objective of this research was to determine and demonstrate the efficacy of soil treatment with ozone in increasing yields in field trial scale applications in a geographically diverse variety of important California crops. Varying application dosages and duration were used in all trials and produce yield and quality from treated plots were compared to those from untreated control plots and, in some cases, plots treated with alternative fumigants. Where applicable, soil pathogen pressures and/or active soil-borne fungi and bacteria populations were determined and correlated with crop yield and treatment.

2.1.2 Project Approach

As a result of the pressing need to develop environmentally benign replacements to methyl bromide for soil fumigation, SoilZone, Inc. commenced field trials using ozone in 1997. This technology use root zone injection of ozone gas that was generated in the field, close to the injection site.

Based on the initial success of these independently evaluated trials involving carrots and tomatoes, SoilZone believed its ozonation technology had the potential to provide a sustainable and long-term alternative to methyl bromide for soil fumigation treatment. SoilZone subsequently requested and received matching research assistance contracts from the Electric Power Research Institute (EPRI) Agricultural Technology Alliance (ATA) and the California Energy Commission through Edison Technology Solutions (ETS) to perform ten field trials in California. Subsequently, field trials were conducted in conjunction with co-investigator Dr. Becky Westerdahl from the University of California, Davis (UCD).

Table 1 lists the crops, California location, research collaborators, and methods of ozone injection for these trials**.**

Table 1. Field Trial Collaborators

Crop	Test Location (California)	Research Collaborator	Ozone Injection Method
Tomatoes	Irvine	Dr. Becky Westerdahl, UCD	Buried Drip Tube
Tomatoes	Tulare	Edison AgTAC/EPRI-ATA	Buried Drip Tube
Carrots	Irvine	Dr. Becky Westerdahl,	Buried Drip Tube
Carrots	Tulare	Edison AgTAC/EPRI-ATA	Buried Drip Tube
Strawberries	Watsonville	Dr. John Duniway, UCD	Buried Drip Tube
Sugar Beets	Irvine	Dr. Becky Westerdahl, UCD	Buried Drip Tube
Broccoli	Santa Maria	Rancho Laguna Farms	Buried Drip Tube
Prune Replant	Orland	Steve Brown, Farmer	Injection Probe
Sweet Potatoes	Stevenson	Nakashima Farms	Buried Drip Tube
Peach Replant	Winton	Mallard Bend Farms	Injection Probe

All ozone injection through drip tubing used 1/2-inch PVC tubing with 12-inch emitter spacing. Tubing was buried 6 inches deep in bed centers except for strawberries where double injection tubes were used for each bed and buried 10 inches from bed edges.

The injection tubing used for the initial application was left in place throughout the duration of the trial and used for subsequent midseason applications of ozone in the Tulare carrot and tomato trials. In the Irvine tests, the same drip tubing was used both for ozone injection and for subsequent irrigation. Ozone for orchard replants was applied through a 1/2-inch steel injection probe with 3/8-inch emitter holes drilled between 8 and 18 inches in depth. A 6-foot square of plastic mulch was laid down over each injection site and sealed around the injector in the center and around the plastic edges with dirt. All applications used ozone produced in air unless otherwise indicated.

Table 2 tabulates the crop, ozone application method, and ozone application rate used for these trials.

Table 2. Ozone Application Methods

Crop, Location	Ozone Injection Method	Ozone Treatments (per acre or tree)
Tomatoes, Irvine	0.5 gallons per hour (gph) with 12 inch drip tube	250 lbs. O_3 with and without pre-irrigation
		250 lbs. O_3 in O_2
		50 lbs. O_3 w with and without 100 lbs. CO_2
Tomatoes, Tulare	4.0 gph with 12 inch drip tube	50 and 250 lbs. O_3
		50 lbs. O_3 with 1 x 25 lbs. midseason
Carrots, Irvine	0.5 gph with 12 inch drip tube	250 lbs. O_3 with and without pre-irrigation
		250 lbs. O_3 in O_2
		50 lbs. O_3 with and without 100 lbs. CO_2
Carrots, Tulare	4.0 gph with 12 inch drip tube	50 and 250 lbs. O_3
		50 lbs. O_3 with 2 x 15 lbs. midseason
		50 lbs. with 100 lbs. CO_2
Strawberries	4.0 gph with 12 inc. drip tube	400 lbs. O_3
		400 lbs. O_3 with 100 lbs. Trichoderma
Sugar Beets, Irvine	0.5 gph with 12 inch drip tube	250 lbs. O_3 with and without pre-irrigation
		250 lbs. O_3
		50 lbs. O_3 with and without 100 lbs. CO_2
Broccoli	2.0 gph with 12 inc. drip tube	50 and 250 lbs. O_3
Prune Replant	Probe Mulch	1.25 lbs. O_3/tree hole
Sweet Potatoes	2.0 gph with 12 inch drip tube	100 and 400 lbs. O_3
Peach Replant	Probe per mulch	1.25 lbs. O_3/tree hole

Plots were laid out in random blocks or in a manner ensuring equal spacing of different treatments from each other to minimize field edge effects. Table 3 tabulates the crop, plot size and number, moisture content of the receiving soil, and the ozone concentration used for this series of field trials.

Table 3. Treatment Block Size and Repetitions

Crop	No. of Repetitions and Plot Size Per Treatment	Application Soil (percent) Moisture	O_3 Concentration (percent w/w)
Tomatoes, Irvine	Six – 20 ft x 34 in	12 – 17	2.7 – 6.0
Tomatoes, Tulare	Six – 20 ft x 34 in	10 – 14	2.7 – 6.0
Carrots, Irvine	Six – 20 ft x 34 in	10 – 14	2.7 – 6.0
Carrots, Tulare	Six – 20 ft x 40 in	17-18	1.6 – 1.8
Strawberries	Three – 20 ft x 52 in	8-11	1.8 – 2.0
Sugar Beets	Six – 20 ft x 34 in	12 – 17	2.7 – 6.0
Broccoli	Six – 30 ft x 38 in	14-16	1.8 – 2.0
Prune Replant	Ten Trees – 20 ft on center	13-18	1.5 – 1.6
Sweet Potatoes	Six – 20 ft x 40 in	11.2	2.5 – 2.8
Peach Replant	Ten Trees – 20 ft on center	8-11	1.8 – 2.0

Field test results, for each of the crops involved, are reported in the following narrative.

2.1.2.1 1998 Irvine Tomato Field Trials

These trials were performed in a field heavily infested with root knot nematodes at the University of California South Coast Field Station in Irvine, California. The research was conducted in conjunction with Dr. Becky Westerdahl, University of California at Davis, Department of Nematology.

Ozone was injected in early July through underground drip tubing buried four to six inches deep in the center of 32-inch furrows. Various combinations of pre- and post-irrigation and application rates were used (Table 2).

Tomato seedlings were planted three weeks after the injection treatment and the total yield and number of root galls were compiled at the end of the September harvest. These results were compared to untreated controls and plots treated with other fumigants.

In these trials, yields from pre-irrigated plots treated with ozone at a rate of 250 lbs. per acre were approximately 44 percent higher than yields from the untreated control plots (Figure 1). The yield differential was equal to the metam sodium-treated plots and 17 percent greater than yields from the Telone-treated plots. Plots treated with ozone at 50 lbs. per acre with 100 lbs. per acre of carbon dioxide produced yields 30 percent greater than that of the untreated controls.

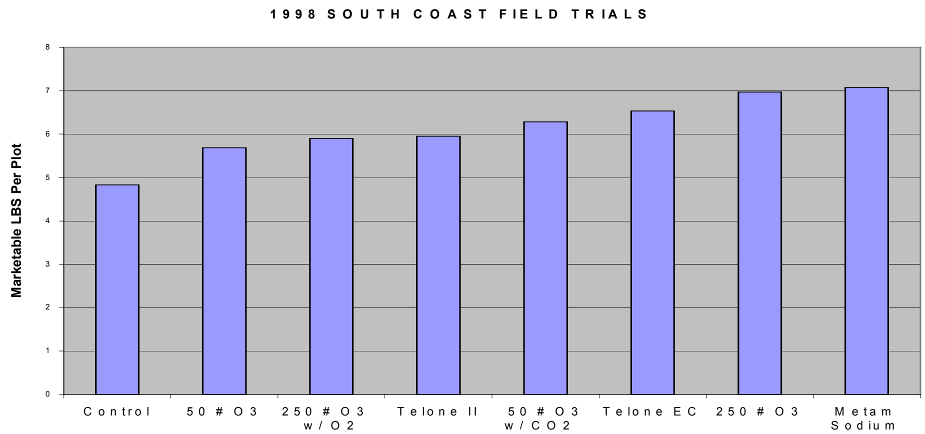


Figure 1. 1998 Irvine Field Trials – Tomato Yield

Nematode root galling was not appreciably lower in the ozone-treated plots than in the Telone-treated control plots despite the improved yields from the ozone treated plots (Figure 2). The increased yield may be the result of biostimulation; probably due to increased soil nutrient availability. These biostimulatory effects, combined with the biocidal aspects of ozone treatment are also important in plant yield.

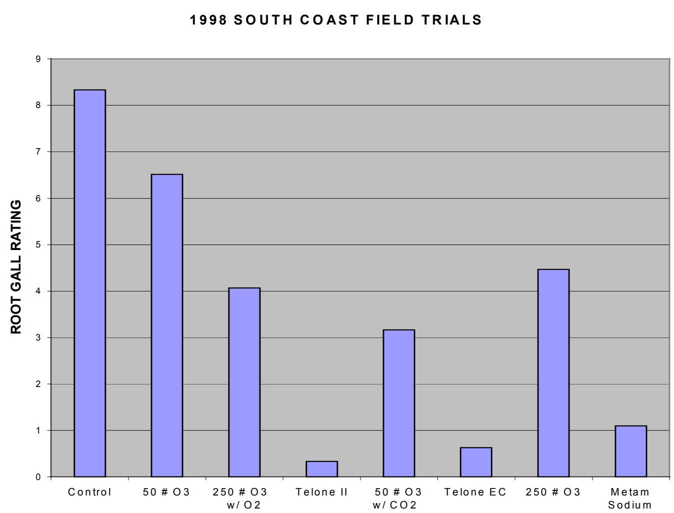


Figure 2. 1998 Irvine Field Trials – Tomato Root Galling

2.1.2.2 1998 Tulare Tomato Field Trials

Field experiments were also conducted during the spring and summer of 1998 at the Southern California Edison Agricultural Technology Application Center (AgTAC) in Tulare, California, in conjunction with the EPRI.

Ozone was injected at the rate of 250 lbs. or 50 lbs. per acre through underground drip lines buried about 6 inches deep in the center of 40-inch furrows.

Tomato seedlings were planted five days after treatment. An additional midseason application of 15 lbs. per acre was applied to half of the 50 lbs. per acre treatments and the total yields were compiled at the end of the harvest.

In these trials, single 50 lbs. per acre preplant ozone-treated plots experienced increased total fruit production of approximately 57 percent compared to untreated controls (Figure 3). Those plots, which also received a 15 lbs. per acre midseason dosage, had total marketable yield increases of 46 percent compared to yields in untreated control plots. The increased production with the absence of any soil-borne pathogen pressures again indicates a bio-stimulative component of soil ozonation.

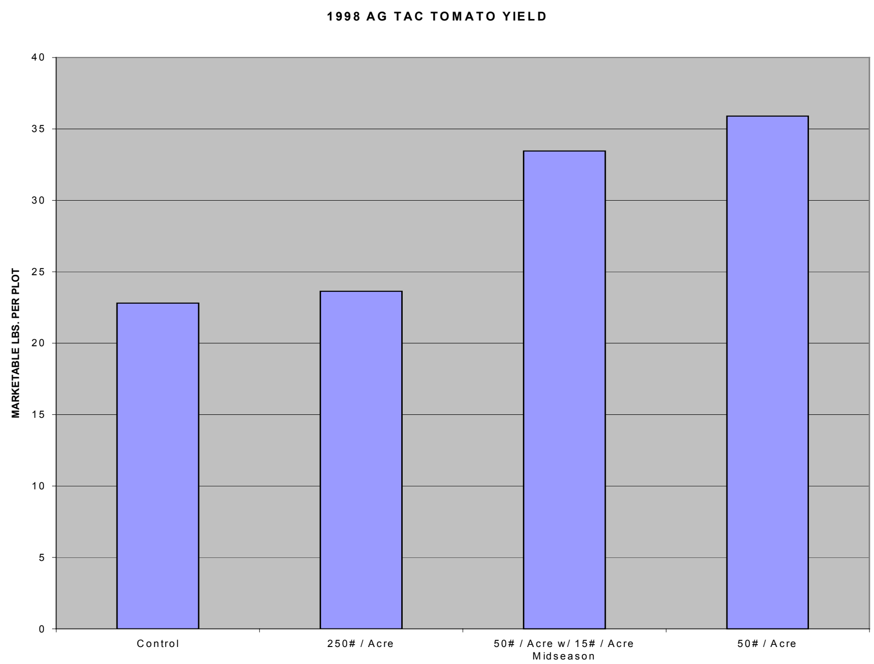


Figure 3. 1998 Tulare Field Trials – Tomato Yield

2.1.2.3 1998 Irvine Carrot Field Trials

These trials were performed in a manner similar to the tomato trials except that carrots were sown from seeds.

Upon harvest, plots that had been treated with either 50 or 250 lbs. per acre of ozone following pre-irrigation experienced a 92 percent increase in total marketable carrot yields compared to untreated controls (Figure 4). Ozone-reated plot production was only slightly less than Telone-emulsifiable concentrate and Vapam treated plots.

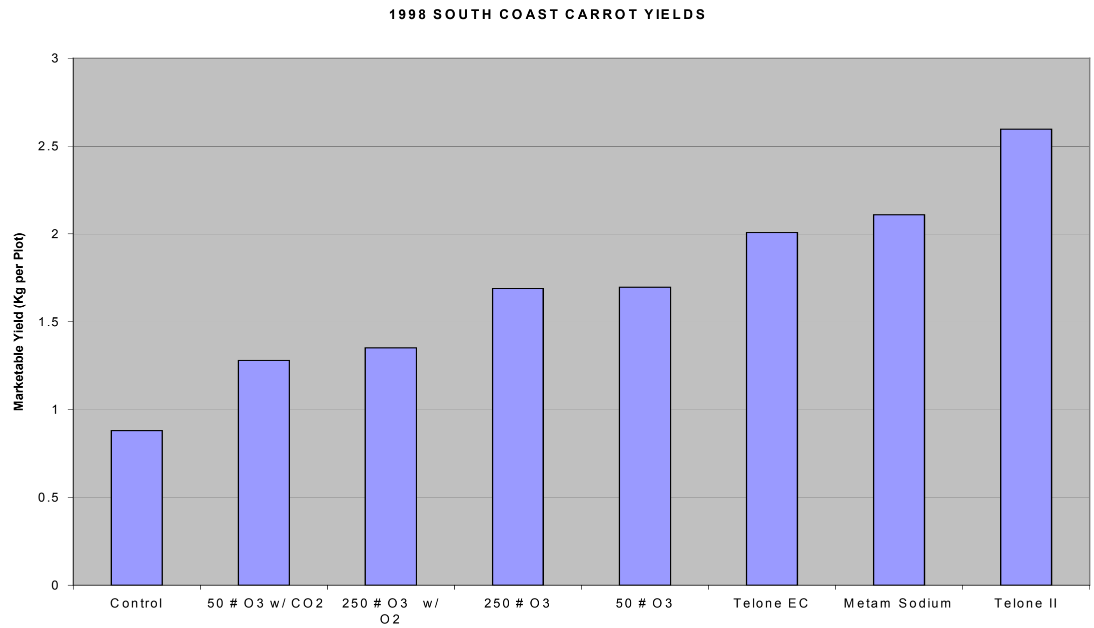


Figure 4. 1998 Irvine Field Trials – Marketable Carrot Yield

The total yield (including nematode damaged produce) was greatest in the 250 and 50 lbs. per acre ozonated plots possibly indicating increased nutrient uptake in these plots (Figure 5).

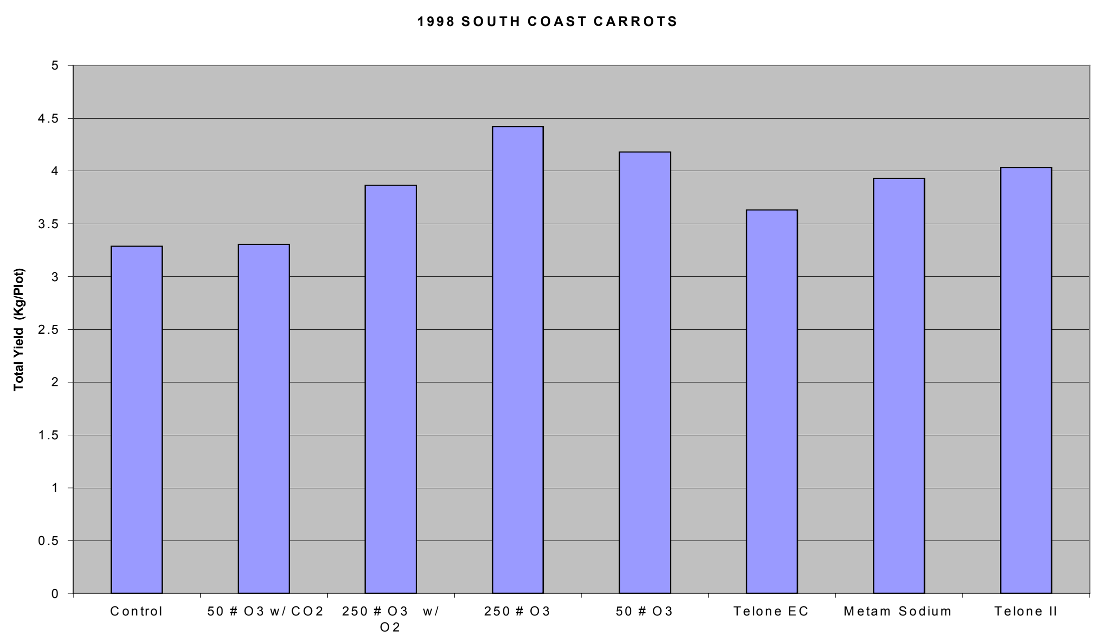


Figure 5. 1998 Irvine Field Trials – Total Carrot Yield

2.1.2.4 1998 Tulare Carrot Field Trials

These field experiments were performed, in conjunction with the EPRI, at the Southern California Edison AgTAC in Tulare, California, during the summer through winter of 1998.

Fields were free of known pathogens. Ozone was injected at the rate of 250 lbs. or 50 lbs. per acre (some co-extensively with 100 lbs. per acre CO_2) through underground drip lines buried about 6 inches deep in the center of 48-inch furrows. Carrot seeds were planted 5 days after treatment. Two additional midseason treatments of 15 lbs. per acre were applied to half of the plot that previously received the 50 lbs. per acre preplanting treatment.

Harvested carrots were segregated into marketable and non-marketable categories and weighed. When the comparison was restricted to similarly seeded and irrigated plots, plots pretreated with 50 lbs. O_3 and 100 lbs. CO_2 produced a 26 percent increase in total marketable carrot yield compared to the untreated control plots (Figure 6). Plots pretreated with 50 lbs. per acre of ozone and two midseason applications of 15 lbs. per acre produced a 15 percent increase in total marketable carrot yields compared to the untreated controls.

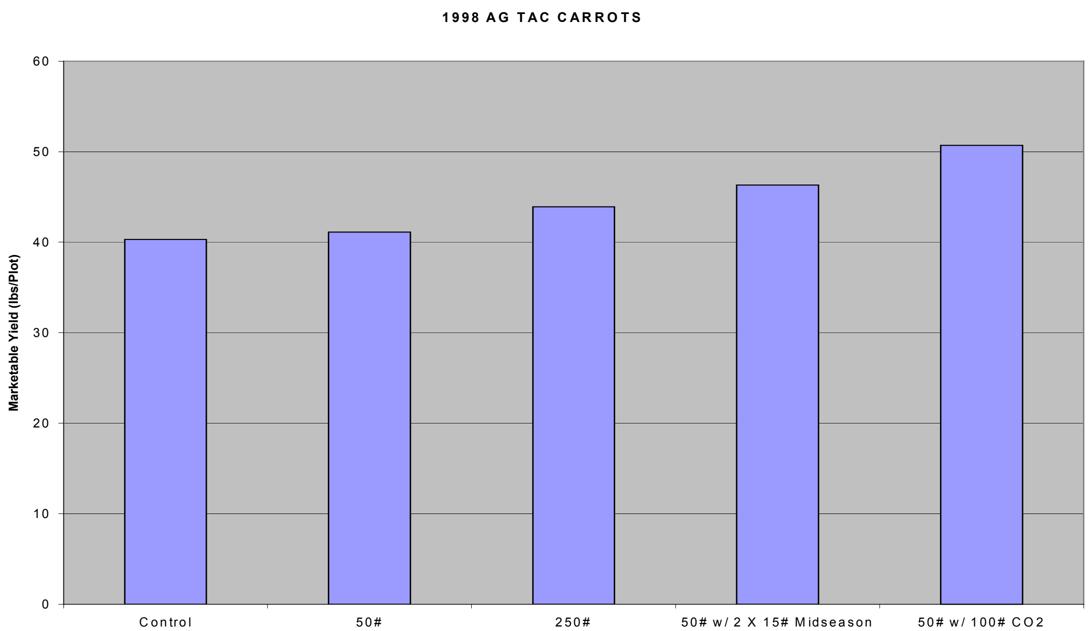


Figure 6. 1998 Tulare Field Trials – Carrot Yield (lbs. per plot)

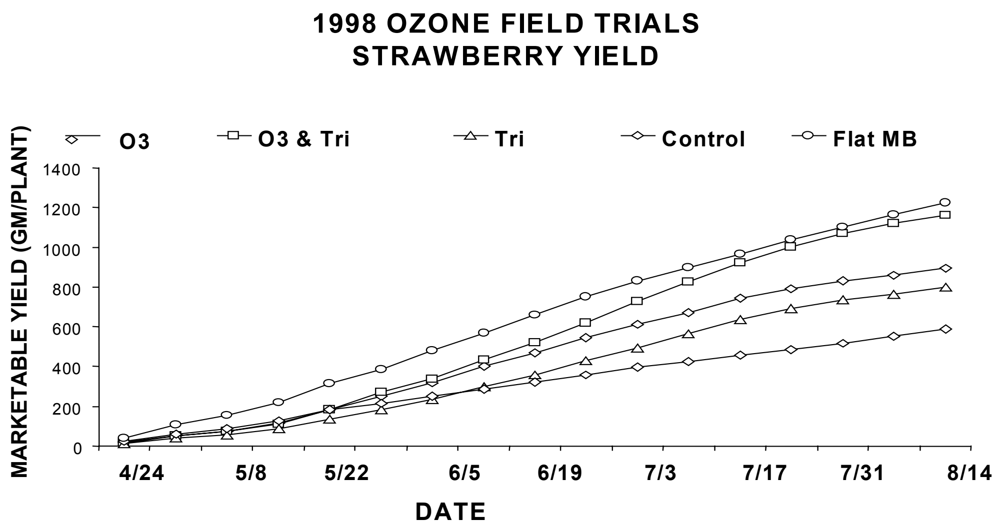
2.1.2.5 Strawberries

This experiment was performed at a site maintained by the U.S. Department of Agriculture (USDA) and the California Strawberry Commission in Watsonville, California, in conjunction with Dr. John Duniway of the UCD Department of Plant Pathology.

The soil at this site was heavily infested with *Verticillium* sp. fungi. Ozone was injected at the rate of 400 lbs. per acre through drip tubing buried about six inches deep in the center of 36-inch beds.

Ozonation applications were made with and without pre-inoculation with Trichoderma fungi. Strawberry transplant planting occurred five days later in November of 1997. In early June 1998, an additional midseason ozone application of 15 lbs. per acre was made to those plots that had been previously inoculated with the Trichoderma sp. fungi.

Cumulative yields totaled through the end of the growing season in early August showed the ozonated plots with Trichoderma fungi produced 97 percent greater marketable fruit than untreated controls (Figure 7). Yields from the ozone-treated plots were functionally equivalent to the methyl bromide/Chloropicrin treated plots. The plots that received ozone only were 51 percent greater than the untreated controls.



Collaborators: Dr. John Duniway, Department of Plant Pathology, UC Davis
Location: Watsonville, CA

Figure 7. 1997-98 Ozone Field Trials – Strawberry Yield

2.1.2.6 Irvine Sugar Beet Trials in Cyst Nematode Infested Soils

These trials were performed in a field heavily infested by cyst nematodes (Hetrodera schachtii) at the University of California's South Coast Field Station in Irvine, California. The research was conducted in conjunction with Dr. Becky Westerdahl of the UCD Department of Nematology.

Ozone was injected in early July both with and without pre- and post-irrigation at the rate of 250 lbs. per acre through underground drip tubing buried four to six inches deep in the center of 32 inch furrows.

Each treatment consisted of six 20-foot rows in randomized complete blocks. Sugar beets (variety HH103) were planted 1 week following the ozone injection and the total yield was compiled at the end of the late December harvest.

In these trials, the best ozone treatment at 50 lbs. per acre increased total sugar beet production by 10.8 percent compared to untreated controls and 13.2 percent compared to Telone treated plots (Figure 8).

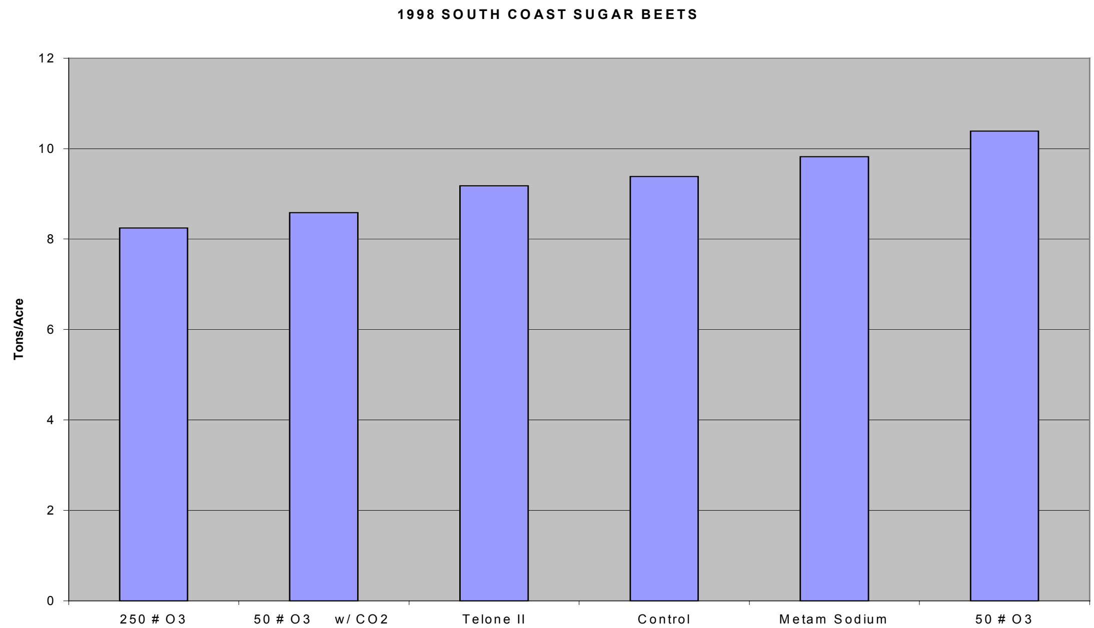


Figure 8. Ozone Field Trials-Sugar Beet Cyst Nematode Infestation

2.1.2.7 Irvine Sugar Beet Trials in Root Knot Nematode Infested Soils

These field experiments were also performed at the South Coast Field Station with Dr. Becky Westerdahl in parallel with the trials described in Section 2.1.2.6 except that plots were placed in soils highly infested with the root knot nematode (Meloidogyne javanica). The methods and dates of applications were identical to those described in Section 2.1.2.6.

In these trials, preplant treatment with 50 lbs. ozone combined with 100 lbs. CO_2 per acre experienced increased total production of approximately 2.5 percent compared to the untreated controls (Figure 9). Yields from the ozone-treated plots were 7.4 percent greater than those from the metam sodium-treated plots. Yields were, however, 20.8 percent less than those obtained from the Telone-treated plots.

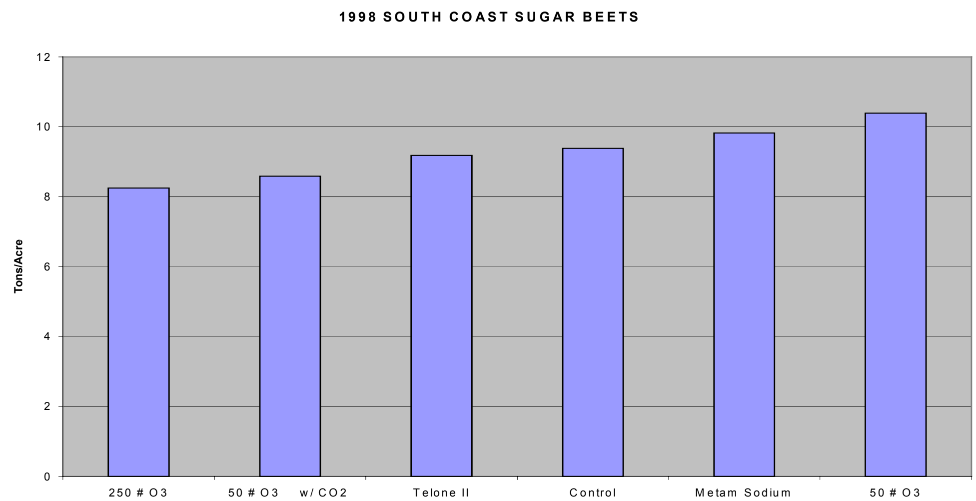


Figure 9. Irvine Field Trials Sugar Beet Root Knot Nematode Infestation

2.1.2.8 Broccoli

These experiments were performed in the summer of 1998 at a private farm in Santa Maria, California.

Ozone was injected through drip lines buried 8 inches deep, at 50 lbs. and 250 lbs. per acre rates, and broccoli seedlings were planted 5 days later. Upon harvest 10 weeks later, broccoli heads were segregated into export or domestic quality. Export heads are highly desirable because they command a 200 to 300 percent price premium over domestic quality heads. The 50 lbs. per acre ozone-treated plots produced a statistically significant 20 percent increase in the number of export quality heads produced compared to the untreated controls (Figure 10).

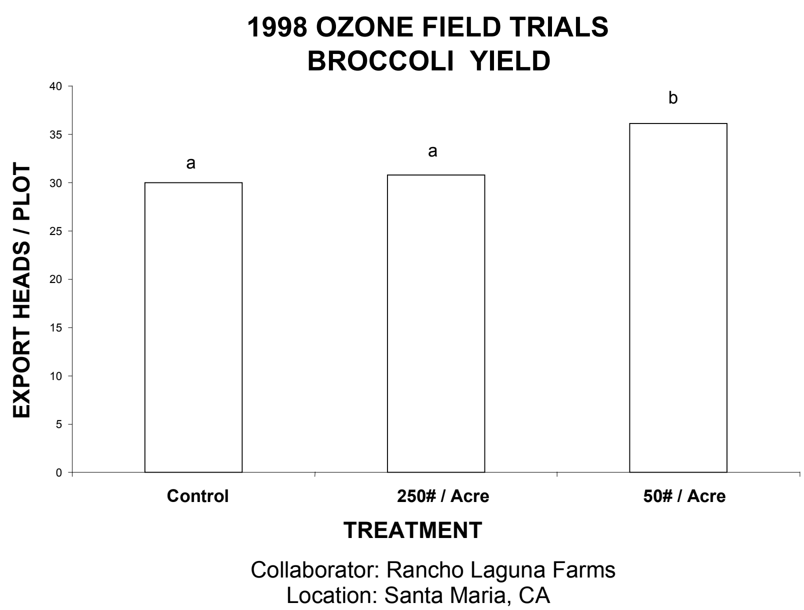


Figure 10. 1998 Broccoli Trials

2.1.2.9 Prunes

These trials were conducted in an established 30-year-old prune orchard with severe lesion and ring nematode population pressures. Tree replant sites were excavated in the fall to remove the stumps and larger roots and the sites were re-mounded. Ozone was applied at the rate of 1.25 lbs. per hole through an injection probe with 3/8-inch emitter holes drilled between 8 and 18 inches in depth. Trees were planted 1 week later in March. The survival rate, tree diameter, and vigor were evaluated in November.

Trees planted in ozone treated holes had a 70 percent survival rate which was equal to or greater than the survival rates for trees grown in Enzone, Telone, and methyl bromide treated soils and in untreated soil (Figure 11).

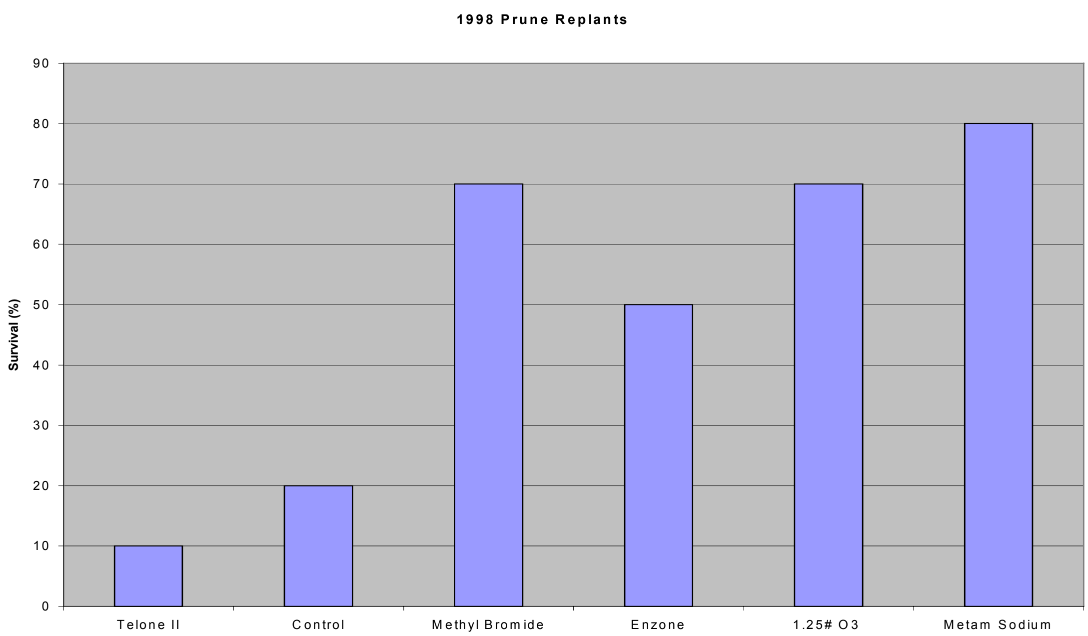


Figure 11. 1998 Ozone Field Trials-Prune Replants Survival

By multiplying the average vigor rating of the surviving trees by the survival percentage, an overall rating of treatment effectiveness is obtained. When so evaluated, trees planted in ozone-treated soil showed an overall rating substantially greater than trees planted in untreated soils or soils treated with methyl bromide, Enzone, or Telone. Metam sodium treated trees exceeded the ozone treatment trees' overall rating only slightly (Figure 12).

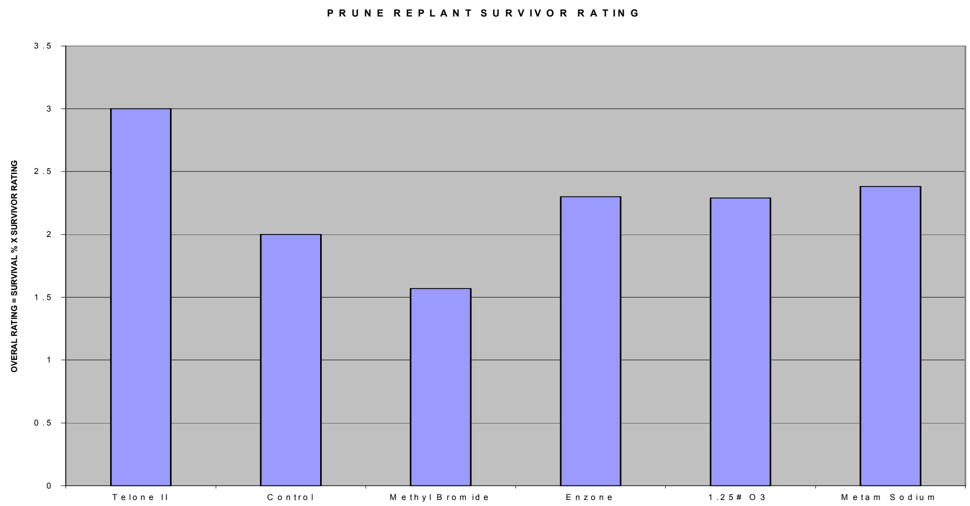


Figure 12. 1998 Ozone Field Trials-Prune Replants Treatment Effectiveness

2.1.2.10 Sweet Potatoes

These experiments were performed in May through October of 1998 at a private farm in Stevenson, California. Ozone injection was performed in early May at 100 lbs. or 400 lbs. per acre through drip lines buried 7 inches deep. Sweet potato seedlings were planted 5 days later.

Upon harvest in early October, potatoes were segregated into large marketable or small potatoes. The 100 lbs. per acre ozone-treated plots produced an average 15 percent increase in large potato weights compared to the untreated controls (Figure 13).

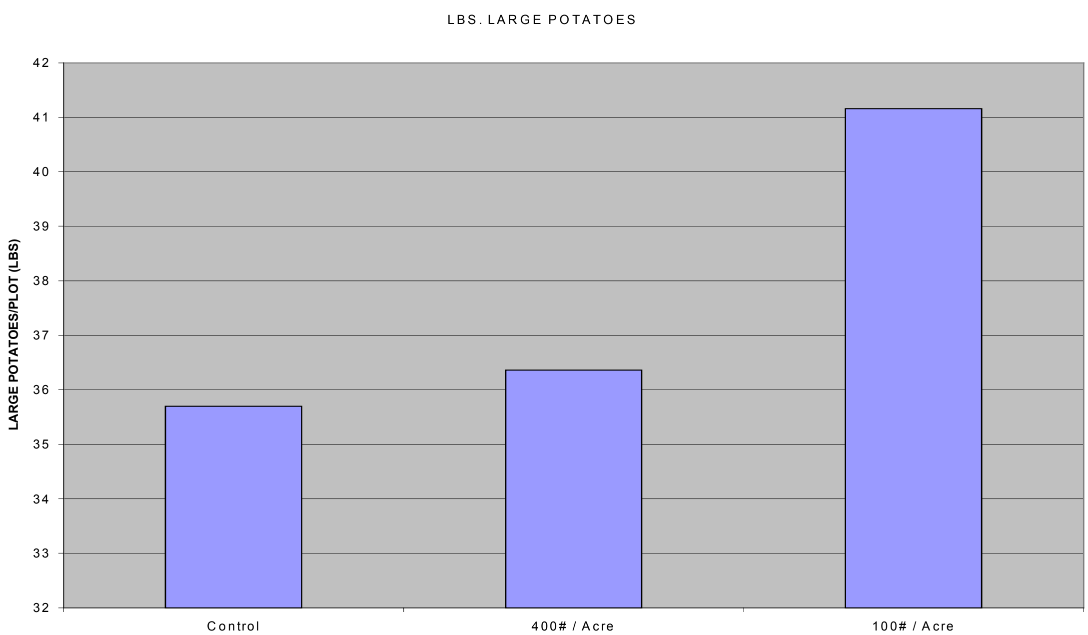


Figure 13. 1998 Ozone Field Trials – Sweet Potato Yield

2.1.2.11 Peaches

These trials were conducted on the non-productive periphery of an established 25-year-old peach orchard. In the same manner as the prune trials, 1.25 lbs. of ozone was injected into an excavated and re-mounded tree site 5 days before trees were planted. The injection probe had eight 3/8-inch holes spaced around the probe at a buried depth of from 6 to 18 inches through which the ozone was injected into the probe. Within several weeks, an apparent phytotoxic effect was noticed in the trees that had been planted in ozonated soil.

The trunks of these trees turned a darker brown and the few leaflets that had formed were very small and very dark green. These are symptoms characteristic of nitrogen burn due to excess ammonia or nitrate nitrogen in the soil. Subsequent soil analysis revealed nitrate nitrogen increased in the ozonated tree holes from 26 parts per million (ppm) to 149 ppm and ammonia nitrogen increased from 2.1 ppm to as high as 16 ppm.

It was concluded the phytotoxicity was probably caused by an excess formation of nitrogenous compounds in the soil resulting from over ozonation. These tests will be repeated with lower ozone dosages in the future. A compounding factor in these trials was that many of the untreated control replants also showed poor vigor. It is believed that this was due to root fungus on the replant roots originating from improper handling after removal of the trees from cold storage. It is not known if similar results would have occurred if all the ozonated tree replants were not similarly afflicted (Figure 14).

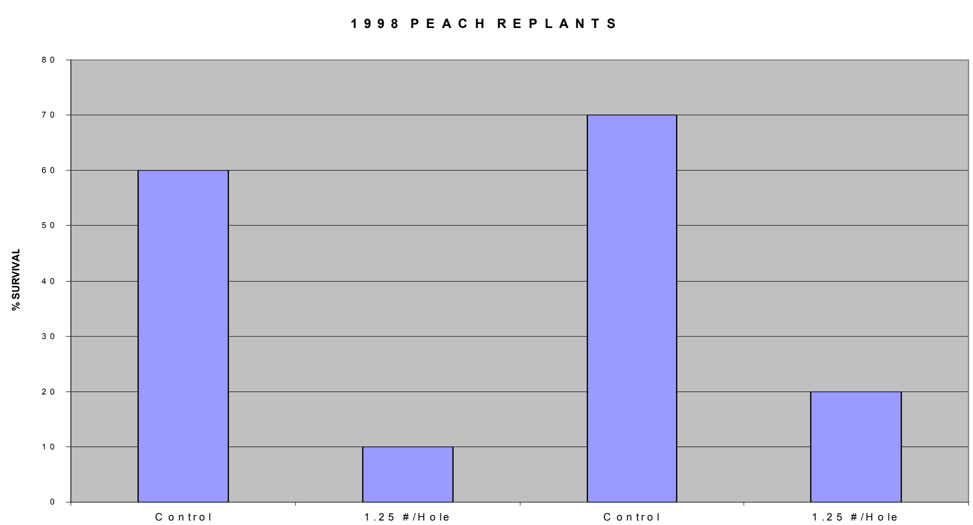


Figure 14. Ozone Field Trials – Surviving Peach Replants

2.1.3 Project Outcome

The results of these field trials generally demonstrated the broad effectiveness of ozone treatment of soil to increase plant yield and reduce the detrimental effects of soil pathogens on a variety of commercially important crops and soil types under a range of climatic conditions. Field test results indicate that soil treatment with ozone decreased soil pathogen pressures (due to ozone's ability to kill living organisms) and increased nutrient availability (due to ozone's oxidative effects on soil organics). Table 4 provides the increase or decrease in yield resulting from ozone treatments at each site compared to untreated controls.

Table 4. Ozone Treatment Crop Yield Compared to Untreated Control

Crop	Ozone Treatments (per acre or tree)	Crop Yield Compared to Untreated Control
Tomatoes, Irvine	250 lbs. O_3 with pre-irrigation	+44.2%
	250 lbs. O_3 without pre-irrigation	+35.1%
	50 lbs. O_3 with 100 lbs. CO_2	+30.0%
	250 lbs. O_3 in O_2	+22.1%
	50 lbs. O_3	+17.6%
Tomatoes, Tulare	50 lbs. O_3	+57.4%
	50 lbs. O_3 with 1x 25 lbs. midseason	+46.7%
	250 lbs. O_3	+3.7%
Carrots, Irvine	50 lbs. O_3	+92.2%
	250 lbs. O_3 with pre-irrigation	+92.0%
	250 lbs. O_3 in O_2	+53.6%
	50 lbs. O_3 with 100 lbs. CO_2	+45.4%
	250 lbs. O_3 without pre-irrigation	-9.1%
Carrots, Tulare	50 lbs. O_3 with 100 lbs. CO_2	+25.6%
	50 lbs. O_3 with 2 x 15 lbs. midseason	+14.8%
	50 lbs. O_3	+8.8%
	250 lbs. O_3	+2.0%
Strawberries	400 lbs. O_3 with 100 lbs. Trichoderma	+96.9%
	400 lbs. O_3	+51.5%
Sugar Beets, Cyst Nematode Laden Soil	50 lbs. O_3	+10.8%
	50 lbs. O_3 with 100 lbs. CO_2	-8.5%
	250 lbs. O_3	+12.2%
Sugar Beets, Root Knot Nematode Laden Soil	50 lbs. O_3 with 100 lbs. CO_2	+2.5%
	250 lbs. O_3	-18.9%
	50 lbs. O_3	-22.6%
Broccoli	50 lbs. O_3	+20.3%
	250 lbs. O_3	+2.8%
Prune Replant	1.25 lbs. O_3/tree hole	+300.7% overall survival/vigor rating
Sweet Potatoes	100 lbs. O_3	+15.3%
	400 lbs. O_3	+1.8%
Peach Replant	1.25 lbs. O_3/tree hole	Control survival: 70% Ozonated survival: 20%

Table 5 provides the increase or decrease in yield resulting from the best ozone treatment at each site compared to the alternative fumigants tested.

Table 5. Ozone Treatment Compared to Alternative Fumigant Crop Yield

Crop	Best Ozone Treatment (per acre or tree)	Crop Yield Compared to Alternative Fumigant Yield
Tomatoes, Irvine	250 lbs. O_3 with pre-irrigation	+17.1% versus Telone II
		-1.4% versus Vapam
Carrots, Irvine	50 lbs. O_3 with pre-irrigation	-20.8% versus Telone EC
		-19.2% versus Vapam
Strawberries	400 lbs. O_3 with 100 lbs. Trichoderma	-9.5% versus Methyl Bromide
Sugar Beets, Cyst Nematode Laden Soil	50 lbs. O_3 with pre-irrigation	+13.2% versus Telone II
		+5.8% versus Vapam
Sugar Beets, Root Knot Nematode Laden Soil	50 lbs. O_3 with pre-irrigation	+7.4% versus Vapam
		-20.8% versus Telone II
Prune Replant	1.25 lbs. O_3/tree hole	Overall survival/vigor rating -15.8% versus Vapam +39.4% versus Enzone +434% versus Telone II 45.8% versus Methyl Bromide

Modest levels of phytotoxicity were noted in the form of lower leaf burn on a number of plants in several plots following midseason applications of only 15 or 25 lbs. of ozone per acre in the tomato and carrot trials in Tulare. The application of ozone in midseason in tomatoes at Tulare resulted in a slightly lower yield than what was harvested from plots not receiving ozonation.

In contrast, the plots which received midseason ozonation applications in the strawberry trials in Watsonville, California, showed substantially increased growth compared to plots that received only a preplant treatment. Further work is needed to properly define the correct dosage levels to maximize yields without phytotoxicity.

The effects of mixing carbon dioxide with the ozone gas for injection as a preplant soil treatment were mixed. In the case of the tomato and sugar beet trials in root knot nematode laden soil in Irvine and the carrot trials in Tulare, the coextensive use of carbon dioxide resulted in increased yield. The opposite effect, however, was seen in the carrot trials and sugar beet trials in cyst nematode laden soils in Irvine.

Numerical (as compared to statistically significant) increases in total fungal biomass were noted in some trials but not in others. The underlying mechanisms of such stimulation are not known and further laboratory work is required in conjunction with field experiments to understand this occurrence.

The economic effectiveness of the ozone treatments is more difficult to ascertain than is the case for conventional agricultural fumigants. Conventional fumigants are sold and delivered to the grower for application by the pound. In the case of soil treatment with ozone, two additional cost components require consideration: the operating cost to power the generation equipment and equipment-related rental cost (including labor, transportation, overhead and capital cost amortization of the ozone producing equipment).

The amortization component of the cost representing 1 lb. per day of ozone generating capacity is extremely variable. It primarily depends on whether the equipment is used on a continuous basis or only intermittently. It is estimated that a service company could provide onsite delivery of ozone for $3 to $4 per lb. with cash margins of approximately 30 percent consistent with industry norms.

Assuming the ozone delivery injection tubing is also used for irrigation during the growing season and that the delivered price of ozone is $3.50 per lb., an applied dosage of 50 lbs. of ozone per acre could be secured for a total price of $175 per acre. This is competitive with the cost of Vapam and Telone. Vapam costs $60 to $80 per lbs. and is applied at a rate of 150 to 200 gallons per acre for a total product cost of $90 to $160 per acre. With application costs at approximately $30 per acre, total costs would be $120 to $190 per acre.

Telone costs about $12 per gallon and about 12 to 14 gallons per acre is used. With an application rate of $50 per acre, this would result in a total applied cost of $194 to $218 per acre. Methyl bromide costs from $3 to $4 per lb. with application rates of from $150 to $300 lbs. per acre. Product costs of approximately $450 to $1,200 per lb. do not include application and tarping costs of $200 to $500 per acre for a net cost of $750 to $1,700 per acre.

A dosage of 250 lbs. per acre of ozone would cost $875 per acre, which is competitive with methyl bromide costs.

2.1.4 Conclusions and Recommendations

Field trial results demonstrated the broad effectiveness of ozone treatment of soil in increasing plant yield and reducing the detrimental effects of soil pathogens in a variety of crops and soil types under a range of climatic conditions. In every trial except the peach trial, substantial improvements in crop yield or plant vigor resulted from the ozone preplant application compared to untreated controls. In many cases where alternative fumigants were also tested, the best ozone treatment often exceeded one or more of the conventional fumigant treatments.

The study concludes that soil treatment with ozone results in decreased soil pathogen pressures (due to its biocidal effects) and increased nutrient availability (due to its oxidative effects on soil organics). Together as a preplant treatment, these benefits promote increased plant growth and yield without detrimental environmental effects.

Much additional work is necessary, however, to enable accurate prediction of the specific growth response achieved by ozonation in difference crops, soil types and climatic conditions.

2.2 Ozone as an Aqueous Disinfectant

In 1997, an expert panel reviewed the safety and potential benefit of ozone use in food processing. The panel declared ozone to be Generally Recognized As Safe (GRAS) for food contact applications (U.S. Food and Drug Administration 1997). The declaration of GRAS status for ozone was submitted to the Food and Drug Administration and its use on food products was legalized in the United States.

Since receiving GRAS status, interest in developing ozone applications in the food industry has increased. In the mid-1990s, ozone was also approved for some food processing applications in Japan, France, and Australia. Ozone has been reviewed for water disinfectant applications[i] and for food processing applications[ii]. Ozone's chemistry has been described[iii], and the practical aspects of the design and operation of ozonators have been reviewed.[iv]

Ozone has been described as an excellent alternative to hypochlorite as a disinfectant or sanitizer, although the two chemicals differ in many aspects (Table 6). Ozone's primary advantages are that in water it decomposes quickly to oxygen, leaving no residues, and has more potency against bacteria, cysts of protozoa, viruses, and fungal spores than hypochlorite.

Table 6. Comparison of Various Aspects of Hypochlorite and Ozone in Water

Attribute	Hypochlorite	Ozone
Microbial potency	Kills plant pathogens and microbial saprophytes effectively. Some human-pathogenic, spore-forming protozoa resistant. Maximum allowable rates under regulatory control.	Kills plant pathogens and microbial saprophytes effectively, including spore-forming protozoa. Maximum rate limited by ozone solubility, difficult to exceed about 10µg/ml.
Cost	Chemical cost low. Repeated delivery required, sometimes pH and concentration controller systems needed, minor maintenance and energy costs, chlorine storage issues.	Variable; no chemical cost, but high initial capita cost for generator, usually needs filtration system when water reused some are complex, modest maintenance and energy costs.
Influence of pH	Efficacy diminishes as pH increases, about pH 8 pH adjustment may be needed. Chlorine gas released at very low pH (4 or less).	Potency not influenced very much by pH, but some decomposition increases at high pH.
Disinfection byproducts	Some regulatory concern, tri-halo compounds, particularly chloroform, of some human safety concerns.	Less regulatory concern, small increase in aldehydes, ketones, alcohols, and carboxylic acids created from organics, BrO3 from OBr.
Worker safety issues	Chloroamines can form and produce an irritating vapor. Chlorine gas systems require onsite safety measures OSHA time weighted average (TWA) limit for chlorine gas: 1 µg/ml.	Offgas ozone from solutions is an irritant and must be managed. MnO_2 ozone destruction efficient and long-lived. OSHA (TWA) limit for ozone gas: 0.1 µg/ml.
Persistence in water	Persists hours in clean water, reduced persistence to minutes in dirty water.	Persists minutes clean water, reduced persistence to seconds in dirty water.
Use rates	Limited by regulation to 25 to 600 µg/ml, depending on application.	Not limited by regulation, but Henry's law limits theoretical maximum ozone in water to about 30 µg/ml. At 20 °C, most ozone systems produce 5 µg/ml, or less in water.
Use in warm water	Increases potency, some increase in vapors.	Not practical, rapidly accelerates ozone decomposition, increases offgassing, decreases ozone solubility.
Influence on product quality	Little risk of injury at recommended rates. Some injury possible above 50 µg/ml, on tree fruits. Off-flavors on some products at high rates.	In brief water applications, risk of product injury low. Stem, calyx, and leaf tissue more sensitive than fruits. Risk of injury needs more evaluation.
Impact on water quality	Minor negative impact: water salt concentration increases somewhat, may interfere with fermentation used to reduce biological oxygen demand, some pesticides inactivated, discharge water dechlorination may be required.	Mostly positive impact: does not increase salt in water, many pesticides decomposed. Biological/chemical oxygen demand may be reduced, flocculation and biodegradability of many organic compounds enhanced, removes colors, odors.
Corrosiveness	High, particularly iron and mild steel damaged.	Higher, particularly rubber, some plastics, yellow metals, aluminum, iron, zinc, and mild steel corroded.

Ozone has also been reported to have a mode of action that controls plant pathogens not solely on anti-microbial activity. Earlier investigators reported that ozone controlled Rhizopus stolonifer and induced resveratrol and pterostilbene phytoalexins in table grapes, and that these made the berries more resistant to subsequent infection.[v] Ozone can oxidize many organic compounds, particularly those with phenolic rings or unsaturated bonds in their structure[vi] and can have a role in reducing pesticide residues.[vii]

The combined wholesale value of California's fresh citrus (oranges, lemons, and grapefruit) and stone (peaches, plums, and nectarines) fruit market is estimated to be $1.6 billion annually. The most important after-harvest pathogens of both citrus and stone fruit are fungi. All packers use methods to limit damage caused by these fungi but losses still average about 5 to 10 percent despite application of today's best available management practices.

The most commonly used fungicides today are hypochlorite, sodium ortho-phenylphenate (SOPP) and sodium carbonate. All these chemicals are either hazardous, toxic, or difficult to handle and transport. There is an urgent need to identify and promote the use of alternative fungicides that are less damaging to the environment and to humans who come in direct contact with fungicides during their application.

Present use of dump-tanks require large amounts of hypochlorite for disinfection. As the solution becomes contaminated rapidly with dirt, the water/hypochlorite mixture is discharged, creating environmental problems. The use of ozone as a disinfectant does not contaminate the water, allowing longer periods before discharging and possible use of the water for other agricultural applications/processes. There are several fruit handling processes where ozone in water applications could be very useful, they include:

- **Ozonation of Dump Tank Water**. The water in tanks where fresh fruit are dumped before cleaning, sorting, and packing operations is an important site for the accumulation of pathogens that infect fruit later in storage, shipping, or marketing. An example is blue mold of apples and pears, caused by *Penicillium expansum*.[viii] Therefore, disinfection of this water is important, and is usually accomplished with hypochlorite.
- **Treatment of Wounded Fruit**. Ozone in water treatment of pathogen wound-inoculated fruit. Many pathogens use fruit surface wounds, inflicted at harvest, to initiate infections that are sometimes expressed long afterward. An example is green mold of citrus, caused by *Penicillium digitatum*. These infections are typically controlled by fungicides that are applied on fruit packing lines.
- **Pathogen Control**. Ozone in water treatment to control pathogens, that do not require wounds on the fruit surface to initiate infections. They can be controlled by fungicide or sanitizer applications. An example is the contamination of grapes by spores of *Botrytis cinerea*, cause of gray mold.
- **Microbe Reduction**. Ozone in water treatment to reduce natural microbe populations. The quality of some products is reduced when natural microbe populations on them are high, although they are saprophytes that do not cause post-harvest decay or comprise a food safety hazard. An example is the preference buyers have for strawberries with low total microbe populations compared to those with high populations, particularly when the strawberries are used subsequently in foods that are not sterilized by cooking.

- **Discharge Water Treatment**. Ozone treatment for discharge water quality purposes. Some facilities have water quality discharge compliance issues that could be alleviated by ozone treatment, including the reduction of pesticide residues, biochemical oxygen demand (BOD), or avoiding increases in water salt content.

2.2.1 Project Objective

Our purpose was to evaluate the use of ozone in water to:

- Kill the spores of the major post-harvest pathogenic fungi
- Reduce surface microbe populations or control post-harvest diseases on various fresh fruit
- Determine the tolerances of the various fresh fruits to different ozone exposures
- Evaluate ozone impact on water quality (BOD reduction, the destruction of the fungicides imazalil, thiabendazole, and sodium ortho-phenyl phenol).

2.2.2 Project Approach

2.2.2.1 Equipment Set-Up and Operation

Ozone in water was tested using a generator, contractor, tank, and filter system leased from TechOzone, Inc. Ozone gas was generated from pure oxygen gas that flowed through a water-cooled, corona discharge unit. The ozone gas was dissolved in water in contractor tanks and the ozonated water pumped continuously through a 1,000-liter (265-gallons) tank and returned to the contractor tanks.

The entire tank volume passed through the ozonator system once every 4 minutes. Ozone gas evolved constantly from the water surface, particularly when high ozone concentrations were tested. To manage the nuisance and hazard this comprised, the tank was enclosed with a cover and fan that passed ozone-containing air through MnO_2 pellets to destroy ozone gas before discharge to the atmosphere.

Ozone concentration in water was continuously monitored with an ozone selective electrode[ix] and did not change significantly during the treatments. The concentration of ozone in water was measured colorimetrically (indigo blue test) with a Hach DR 890 colorimeter to calibrate the ozone selective electrode and verify the ozone concentration periodically. In some tests, the pH of the ozonated water was maintained by the addition of 2 mM Na_2HPO_4 and adjusted by the addition of concentrated H_2SO_4 or KOH.

Seven fungi, all important post-harvest pathogens of fresh fruit or vegetables, were cultured from one to 2 weeks at 25 °C on potato dextrose agar. They included *Penicillium digitatum* (isolates M6R and 1165), *Penicillium italicum* (isolate 99-1), *Rhizopus stolonifier* (isolate 95-3), *Botrytis cinerea* (isolate 93-58), *Penicillium expansum* (isolate KBA99-1), *Geotrichum candidum* (isolate 93-49) and *Monilinia fructicola* (isolate 79-1).

Small volumes of sterile water were added to each plate to prepare spore suspensions, and the surface of the agar was rubbed with a glass rod. The solution was poured through two layers of cheesecloth and adjusted to 1×10^6 spores per milliliter with a hemocytometer. A 0.2 ml aliquot of the spore suspension was placed on a 3-µ pore size, 2 centimeter (cm) diameter sterile filter, clamped onto a porous glass support. The water was removed from the suspension by the brief application of a low-pressure vacuum to the supported filter.

Ozone solution from the ozone in water system previously described flowed from the ozonated water tank through the filter at a rate of 1.6 milliliter (ml)/second. At the end of each exposure period, 3 ml of 1,000 microgram (µg)/ml calcium thiosulfate was added to destroy any remaining ozone, followed by 5 ml of sterile water. Then the excess water was removed by low-pressure vacuum.

Finally, the filter was removed from the support, inverted, and placed on potato dextrose agar, where most of the spores were deposited. The proportion of germinated spores was determined between 14 and 24 hours later by examination of the agar surface with a compound microscope (100 to 400X).

2.2.2.2 Control of Gray Mold on Table Grapes

The effectiveness of ozone in water to control gray mold on grapes was assessed on single, inoculated berries. Grape berries were cut from the cluster rachis and randomized into three replicates of 50 berries each.

The surface of the berries were inoculated by spraying a suspension of spores of *Botrytis cinerea* (approximately 12,000 spores per ml) about 2 hours prior to treatment by immersion in a solution of ozone (10 ±1 µg/ml, 20 ±1 °C) for one to 6 minutes.

Ozone efficacy was compared to that of sodium hypochlorite (20 and 200 µg/ml total $NaOC_l$), sodium bicarbonate (0.5M, pH 8.2), and ethanol (60 weight/volume) by dipping 50 inoculated berries per replicate in 1-liter solutions of each for 1 minute at ambient temperature. After treatment, berries were placed on metal racks within plastic boxes and stored at approximately 95 percent relative humidity at 15 °C for seven days, when the number of infected berries was counted.

2.2.2.3 Control of Citrus Fruit Post-Harvest Pathogens

Oranges and lemons that had been commercially harvested no more than 2 days before use were randomized and inoculated with *P. digitatum* 24 (±2) hours before treatments were applied. The inoculation method employed simulates infections that occur under commercial conditions and has been recommended for determining the effectiveness of fungicides[x]. *P. digitatum* and *Geotrichum candidum* were cultured 1 to 2 weeks on potato dextrose agar and 5 ml of water containing 0.05 Triton X-100 was added to the dish. Spores were rubbed from the surface with a sterile glass rod, the suspension was passed through two layers of cheesecloth, and adjusted to an absorbance of 0.1 at 425 nanometers (nm). Spore solutions of this density contain about 10^6 spores per ml.

The *G. candidum* inoculum contained 10 µg/ml cyclohexamide and 100 µg/ml thiabendazole to facilitate infection. Fruit was inoculated by immersing a stainless steel rod with a probe tip 2 millimeters (mm) long and 1 mm wide into the spore suspension and wounding each fruit once. The temperature of the fruit at the time of inoculation and treatment was 20 ±1 °C.

Ozone in water at various concentrations was tested. In one test, to control post-harvest green mold on lemons, sodium bicarbonate was added to a concentration of 3 weight/volume (wt/vol) to the water and the pH adjusted to 7.5 before ozonation. After treatment, the fruit was placed into plastic cavity trays to prevented accidental contact infections and stored at 13 °C, a common storage temperature for citrus fruit, or 20 °C, a common temperature during transportation or in retail trade for citrus fruit, for up to 1 month when the incidence of green mold was determined.

2.2.2.4 Influence on Natural Microbe Populations on Harvested Strawberries

Strawberries were hand-harvested by commercial picking crews and randomized just before use. For each treatment, 1 kilogram (kg) (2.2046 lbs.) of strawberries were treated in a 36 cm (14.2 inch) diameter steel mesh baskets. Five replicates of each treatment were completed.

Strawberries were immersed in both chlorinated and ozonated water and the populations of yeast and molds and mesophilic anaerobic bacteria were determined. The strawberries were treated in a solution of 0.5 to 1 µg/ml or 4 µg/ml ozone in water (T= 16-20 °C; pH = 7.6-8.1) for periods of 10 seconds or 2 minutes.

The berry pulp temperature was about 18 °C. To maximize berry contact with the solutions, strawberries were placed on the bottom of the basket no more than two berries in depth, and the basket was turned repeatedly during treatment.

Ozone treatment was compared to sodium hypochlorite that was applied by immersion of the strawberries for 10 to 12 seconds in a flume containing 15 to 20 µg/ml free sodium hypochlorite. This treatment methodology was used routinely at the facility where this test was conducted. The average total hypochlorite concentration of the flume water was 21 ml (16 to 25 µg/ml) according to the Diethyl-p-phenylinediamine (DPD) method. From each treatment, five 1,000 gram (3.5 oz.) samples were collected and placed in sterile zip-lock bags, then placed on ice before the microbe population procedures are applied.

Samples of 1 kg (2.2 lbs.) each were placed on ice or at 0 to 1 °C and processed within 48 hours. Standard microbiological methods[xi] were used to identify and quantify microbial populations. Two 500 g (1.75 oz) berry samples containing 300 ml buffer each were macerated for 20 seconds at low speed and combined in a 2-liter beaker, mixed with a sterile glass rod, then 1 g was diluted in series in five sterile glass test tubes containing 9 ml of phosphate buffer.

A 0.2 ml aliquot of the macerate from each test tube in the dilution series were plated in one each of two agar media: dichloran-rose bengal-chloramphinicol agar base (DRBC) to suppress the spreading growth of molds and most bacteria and facilitate enumeration of filamentous fungi and yeast; and aerobic plate count agar (PCA) to preferentially favor the growth of mesophilic anaerobic bacteria.

PCA was incubated at 37 °C, instead of 20 °C, and triplicate plates of DRBC and PCA were used. Colonies on PCA plates were counted after 2 days incubation, the DRBC plates were counted after 4 days incubation, then rechecked after three more days.

2.2.2.5 Impact of Ozone on Water Quality

The influence of ozone on microbe populations, BOD, and total organic carbon (TOC) was assessed by standard methods. The ozonation system was operated for 12 hours after the last of the strawberry treatments were applied. The ozone concentration during this period was 4 µg/ml. Periodically, samples were withdrawn and microbe populations, BOD, and TOC were determined.

2.2.2.6 Fungicide Degradation in Ozonated Water

The fungicides imazalil (44.6 active ingredient (a.i.)); Fungaflor 500EC, R&D Packing Services, Ontario, California), thiabendazole (98.5 a.i.; Sealbrite Thiabendazole, EcoScience Corp., Orlando, California), and sodium ortho-phenyl phenate (24.0 a.i.; Freshgard 5, FMC Corporation, Riverside, California) were added simultaneously to the ozone in water system previously described and the water was analyzed to determine their persistence.

To the tank of the ozone system, 44.8 g, 20.3 g, and 83.3 g. of imazalil, thiabendazole, and sodium ortho-phenyl phenate, respectively, were added. Imazalil, thiabendazole, and sodium ortho-phenyl phenate were measured at 17.5, 6.4, and 8.6 µg/ml, respectively, in the water before ozonation. These rates simulate typical concentrations found in discharge water from packinghouses. The water temperature was 18.4 °C and the pH was 6.1.

After mixing for 1 hour without ozone, an initial sample was taken, the ozonator started, and subsequent samples retrieved at 0.5, 1, 2, 4, and 8 hour intervals. Imazalil was analyzed by extraction of the sample with ethyl acetate, followed by gas chromatography (carrier gas: argon 95 percent, methane 5 percent electron capture detector). Thiabendazole content was determined by ultraviolet absorbance of an acidified sample at 302 nm. Sodium ortho-phenyl phenate residual was analyzed by extraction of the sample with hexane, followed by gas chromatography (carrier gas: helium; flame ionization detector). Minimum detection limits of imazalil, thiabendazole, and sodium ortho-phenyl phenate in the tank water were 0.01, 0.1, and 0.1 µg/ml, respectively.

2.2.2.7 Statistical Analysis

The concentrations of ozone that caused 50 percent and 95 percent mortality of spores and the upper and lower 95 percent fiducial limits were estimated by Finneyís Probit analysis. The incidence of decayed fruit was determined by an analysis of variance applied to the square root of the arcsine of the proportion of infected or injured fruit, followed by Fisherís Protected least significant difference (with probability as less than or equal to 0.05) to separate means. Actual values are shown in the following figures and tables.

2.2.3 Project Outcome

2.2.3.1 Mortality of Pathogen Spores in Ozonated Water

Ozone killed spores of all the post-harvest pathogens tested rapidly and effectively. A contact time of 2 minutes in 1.5 µg/ml ozone was required to kill 95 to100 percent of all eight fungi tested, and none survived 3 minutes of contact (Figure 1). Our results agree with those of Spotts and Cervantes (1992[xii]), who reported similar results with three fungi. Ozone doses that killed fungal spores were much higher than those that killed food-related organisms, such as *Salmonella typhimurium, Pseudomonas aeruginosa Listeria monocytogenes, Enterococcus faecalis,* and *Candida albicans*, and slightly lower than the ozone dose that killed cysts of protozoa, such as *Cryptosporidium parvum* (White 1992, Peeters et al 1989). Ozone effectiveness in water to kill spores of *P. digitatum* was not influenced by pH (Figure 16) and could be predicted by a dose and time relationship (Figure 17).

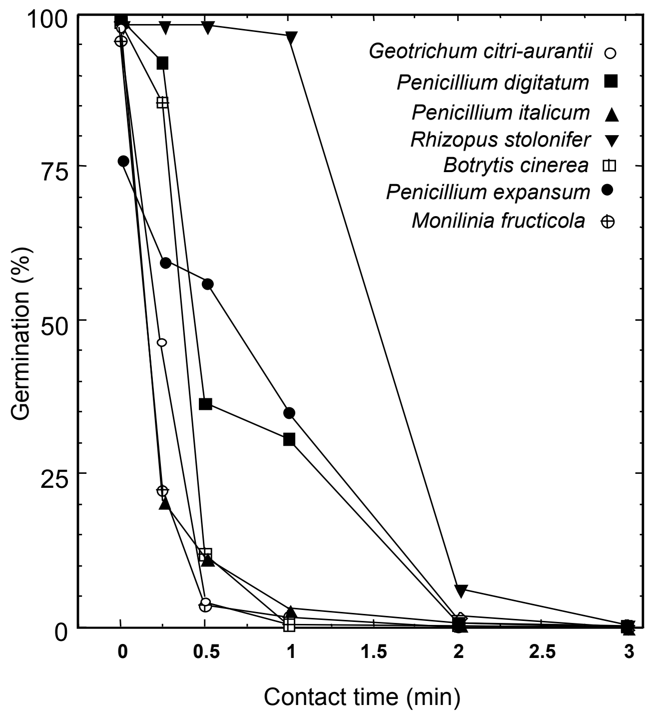


Figure 15. Germination of Post-harvest Pathogenic Fungi

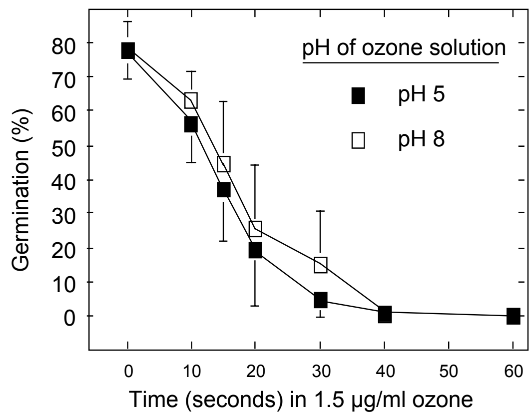


Figure 16. Germination of Spores After Expose to Ozone Solution

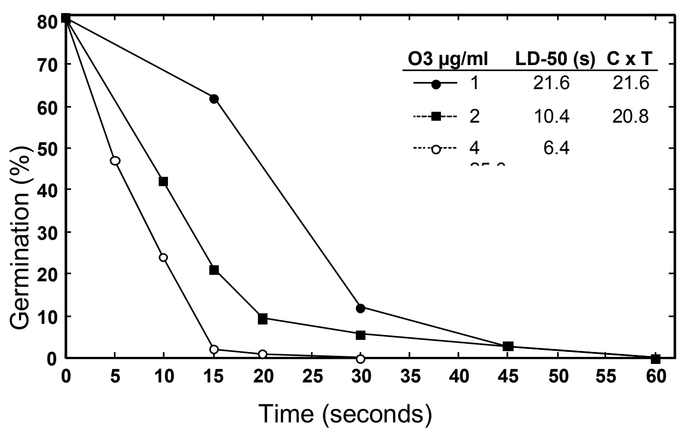


Figure 17. Germination of Spores After Expose to Ozone Concentrations

2.2.3.2 Control of Microbes on Fruit Surfaces

Higher doses of ozone were required to kill pathogens on fruit surfaces than those needed to kill spores in water. A contact time of ten seconds in an aqueous solution of four ml ozone reduced yeast and mold and aerobic bacteria populations on strawberries by 50 percent, while a contact time of two minutes reduced populations about 90 percent (Table 7). The one µg/ml ozone solution was less effective than a solution containing four ml. At 1.5 µg/ml, ozone in water killed 50 percent of *Botrytis cinerea* spores in approximately 25 seconds, while on grape berry surfaces an immersion in a solution of 10ug/ml ozone in water for one to two minutes was needed to reduce the disease about 50 percent (Figure 18). Our results are similar to those of Scherm and coworkers (1993).[xiii] They found ozone at 3.8 µg/ml killed sporangia of the *Bremia lactucae* in water in one minute, while on lettuce leaves protected the sporangia; 25 minutes of ozone at this concentration did not reduce sporangia viability.

Table 7. The Mean Colony-Forming-Units (CFU) per Gram Fresh Weight of Strawberries

Each value is the mean colony-forming units per gram of the five 1000-gram replicates.

Treatment	Active µg/ml	Bacteria CFU			Yeasts and Molds CFU		
		Actual	Log-10	%-Red[a]	Actual	Log-10	%-Red.
Early season							
Control	0	69,813	(4.84)	…	242,240	(5.38)	…
NaOCl, 12 s [b]	25	2,312	(3.36)	97	61,173	(4.79)	75
O3, 10 s	4	8,245	(3.92)	88	63,840	(4.81)	74
O3, 2 min	4	7,750	(3.89)	89	29,224	(4.47)	88
Mid-season							
Control	0	263,026	(5.42)	…	537,032	(5.73)	…
NaOCl, 12 s	16	65,565	(4.81)	75	298,667	(5.46)	44
O3,10 s	4	165,959	(5.22)	37	234,423	(5.37)	56
O3, 2 min	4	24,547	(4.39)	91	47,863	(4.68)	91
Late-season							
Control	0	338,844	(5.53)	…	1,023,293	(6.01)	…
NaOCl, 12 s	21	100,000	(5.00)	71	301,995	(5.48)	71
O3, 10 s	1	141,253	(5.15)	58	588,844	(5.77)	43
O3, 2 min	1	56,234	(4.75)	83	269,153	(5.43)	74
O3, 10 s	4	44,668	(4.65)	87	316,228	(5.50)	69
O3, 2 min	4	11,749	(4.07)	97	64,565	(4.81)	94

[a] %-Red. = Percent reduction in CFU compared to the control.

[b] 10-12 sec contact in flume water, total NaOCl by DPD method.

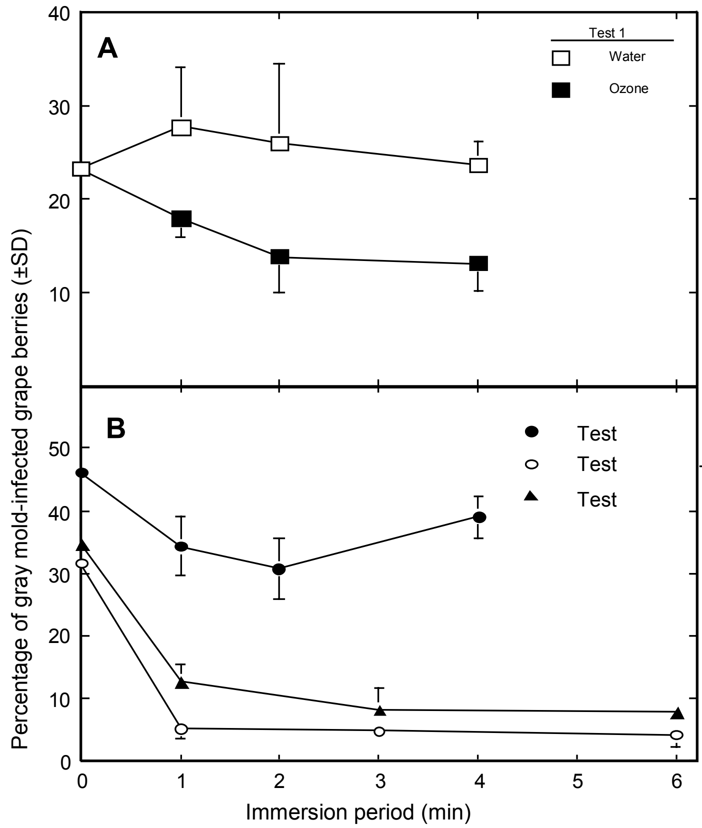


Figure 18. Effectiveness of Ozone in Water for the Control of Gray Mold on Grapes

Past work has shown chlorine sanitizers kill microbes more effectively in solution than on fruit surfaces, and our research suggests this conclusion also applies to ozone in water. For example, Eckert and Eaks (1989)[xiv] reported that 53 ml free sodium hypochlorite at pH 8 killed 50 percent of *P. digitatum* spores in 20 seconds, while on the surface of lemon fruit, 470 ug/ml was required for 50 percent mortality. In a similar study, Brown and Wardowski (1984)[xv] reported that 100 µg/ml total hypochlorite at pH 7 in water killed 100 percent of *G. candidum* spores in 10 seconds, while on the surface of orange fruit, 1,000 µg/ml for 15 seconds caused only 57 percent mortality of the spores.

Ozone significantly reduced gray mold incidence on table grapes, but its efficacy was irregular (Figure 18). In Test 1, water treatment alone did not influence gray mold incidence, while the addition of ozone to the water reduced gray mold about 50 percent. In Test 2, control of gray mold by ozone in water was poor, while in Tests 3 and 4 control of gray mold was excellent. Compared to other treatments, ozone was inferior to sodium hypochlorite, sodium bicarbonate, and ethanol (Figure 19).

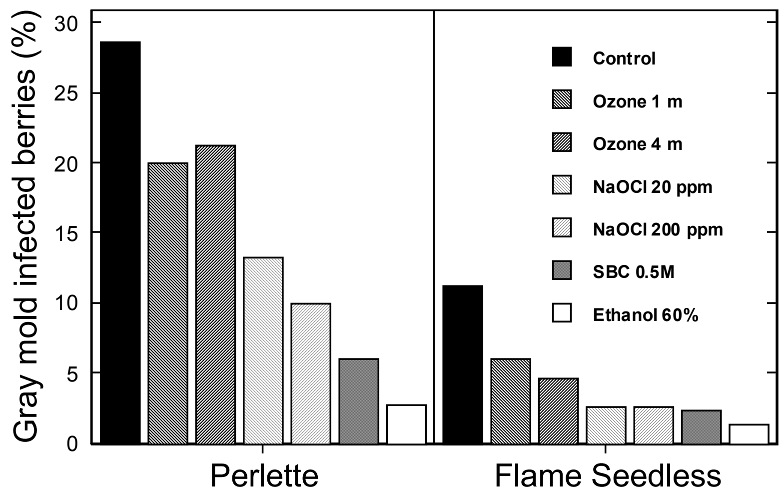


Figure 19. Effectiveness of Ozone in Water and Other Treatments

Compared to the inoculated, untreated control, ozone significantly (orthogonal contrast, P = 0.0227) reduced gray mold, although comparison among individual ozone treatments did not indicate gray mold was significantly (Fisher's Protected least significant difference, P = 0.05) reduced. Prolonged ozone treatment (from 1 to 4 minutes) did not improve its efficacy. The least decay occurred after ethanol treatment, followed by sodium bicarbonate, sodium hypochlorite, and ozone.

Ozone, ethanol, and sodium hypochlorite (20 µg/ml) dip treatments did not injure the berries visibly, while sodium hypochlorite at 200 µg/ml darkened the pedicels of the berries and sodium bicarbonate darkened pedicels and caused some brown spots on the berries. In prior tests, bicarbonate and sodium hypochlorite used as a spray were effective (Mlikota and Smilanick 1998)[xvi] and did not injure the berries visibly.

Ozone effectiveness was irregular and dependent on grape berry condition. It is suspected that cracks on the berry surface visible on Thompson Seedless berries in Test 2 (Figure 19) or around the pedicle of Perlette berries (Figure 20), may have protected spores from the ozone. Most gray mold infections on Perlette grapes originated from cracks near the pedicel, while Flame Seedless berries in the same test had few injuries near the pedicel and infections did not originate there. Alternatively, in cases where effectiveness of ozone was poor, latent infections that began before harvest may have been present on the berries before our inoculation.

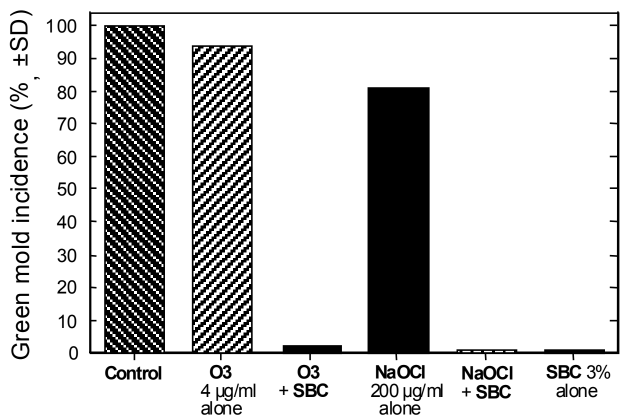


Figure 20. Effectiveness of Ozone and Other Treatments on Citrus Green Mold

2.2.3.3 Control of Pathogens Placed in Wounds on Fruit

The control of pathogens inoculated into wounds on citrus fruit failed even after prolonged treatment with very high ozone concentrations in water (Figure 20), although their spores were killed very quickly in ozonated water (Figure 15).

In preliminary tests, the incidence of green mold on oranges and grapefruit inoculated with spores *P. digitatum* and treated water alone or water with 12 µg/ml ozone for five minutes at 20 °C (pH 7.2) was 100 percent. Similarly, the incidence of sour rot on oranges and grapefruit and inoculated with spores *G. candidum* and treated with water alone for five minutes was 54 percent, while the sour rot incidence among those treated for five minutes with 12 µg/ml ozone was 78 percent.

Similar results were obtained with lemons, even when the ozone contact period was increased to 20 minutes. Apparently, these pathogens were even more protected from ozone than those that resided on the product surface, presumably because of reduced ozone penetration into the wound, the leakage of ozone-reactive substances that reduced ozone dosage inside the wounds, or antioxidants that protected the pathogens. Clearly, disease control efficacy of ozone cannot be predicted by toxicity of ozone to pathogens in water. The inability to control infections on inoculated citrus fruit with ozone treatment in our tests agrees with the results of Spotts and Cervantes[xvii] (1992) in their work with ozone in water treatment of pears.

Like ozone, hypochlorite was similarly ineffective for the control of pathogens in wounds in our tests. Similarly, prior work with hypochlorite and chlorine dioxide at practical concentrations (200 µg/ml or less) showed they did not control infections within inoculated wounds on citrus (Eckert and Eaks 1989; Smilanick et al 1999[xviii]; Smilanick, unpublished[xix]) or pear (Spotts and

Peters 1980[xx]) fruit. Apparently, like ozone, the compounds are too reactive and not sufficiently selective to inactivate spores situated in fresh wounds in the fruit.

Presumably the wounds on fruit are poorly penetrated by ozone because of natural antioxidants or the leakage of reactive substituents from the fruit that consumed the ozone. Ethanol controlled gray mold (caused by *Botrytis cinerea*) on grapes in the present work. It is a less reactive sanitizer and disinfectant, and was successful at controlling other diseases, including gray mold on strawberries and brown rot (caused by *Monilinia fructicola*) on stone fruit, especially if it was heated (Margosan et al 1997[xxi]) or combined with other fungicides (Feliciano et al 1992).

Ethanol is not used commercially for these purposes. Another class of infections are those that occur in the field, which are not associated with wounds, and are protected within the host tissue at harvest. These infections, generally less common and less important that those of wound-requiring pathogens, probably cannot be controlled by ozone or other sanitizers, but can be controlled by some fungicides or heat treatments. Examples include stem end rots of citrus, caused by *Alternaria* spp. and other pathogens, and brown rot of citrus, caused by *Phytophthora* spp.

2.2.3.4 Influence of Ozone Treatment on Fruit Quality

Table grapes, citrus fruit, and strawberries were not visibly injured by the ozone treatments evaluated. The strawberries probably imbibed water during the longer treatments, and some soluble solids were released into the ozone solution. Long (greater than 30 minutes) treatment of oranges in 10 µg/ml ozone caused some flecks of wax to appear on the surface of the fruit, indicating some decomposition of the wax had occurred.

2.2.3.5 Influence of Ozone Treatment on Water Quality

In strawberry wash water, ozonation (4 µg/ml) for three hours greatly reduced microbe populations, moderately reduced BOD, chemical oxygen demand (COD), and suspended solids (SS), did not reduce TOC, and increased total dissolved solids (TDS) (Table 8).

Table 8. Influence of Ozone on Strawberry Wash Water Quality

The ozone concentration during this period was 4 µg/ml and the water temperature was 20 °C.

	CFU/ml Water[a]			mg/L					
Sample	**APC**	**Yeast**	**Mold**	**BOD[b]**	**COD[c]**	**TOC[d]**	**TDS[e]**	**SS[f]**	**pH**
Initial	126,700	9,100	1,000	140	700	140	700	400	7.4
1 hr	…	…	…	120	590	160	940	260	7.2
2 hr	1,030	50	50	94	400	140	900	230	7.1
3 hr	38	5	5	…	…	…	…	…	…

[a] Colony forming units per milliliter of water. APC = aerobic plate count agar.
[b] BOD = Biological/biochemical oxygen demand
[c] COD = Chemical oxygen demand
[d] TOC = Total organic carbon.
[e] TDS = Total dissolved solids.
[f] SS = Suspended solids.

Ozone oxidized imazalil, thiabendazole, and sodium ortho-phenyl phenate very rapidly (Table 9). More than 95 percent of all three were destroyed within 30 minutes. All of these fungicides have phenolic rings or unsaturated bonds in their structure that react very rapidly with ozone (Razumovski and Zaikov, 1984[xxii]). Ozone could be used to reduce the residues of these products in recirculated water or for water quality compliance purposes where they are banned from water discharge ponds or sewers. Analysis of the decomposition products of each fungicide should be conducted. Oxidation products from most organic compounds are innocuous, but in a few cases, they may be more toxic than the original compound (Nickols and Varas 1992)[xxiii].

Table 9. Persistence of the Fungicides in 2000 L of Ozonated Water

Time	Ozone produced (g)	Grams in 2000 L of Water		
		Imazalil	Thiabendazole	SOPP[a]
0	0	50.30	20.0	20.3
0.5	170.5	2.40	0.0	1.1
1	340.5	0.52	0.0	0.0
2	681.0	0.40	0.0	0.0
4	1362.0	0.06	0.0	0.0
8	2724.0	0.00	0.0	0.0

[a]SOPP = sodium ortho-phenyl phenate

2.2.4 Conclusions and Recommendations

This work is still in progress and it is premature to declare conclusions on many aspects of the project. Ozone clearly has shown promise, however, as a sanitizer to minimize the chemical and microbial contamination of water within dump-tanks, flotation solutions, brush bed or high pressure washers, or other process water that contacts the fruit during post-harvest handling. Sanitation of fruit surfaces can be achieved, but contact times must be long compared to other sanitizers, and the ozone concentration must be high. It could replace hypochlorite for the control of gray mold, but probably with some loss of efficacy.

Ozone is compatible with bicarbonate salts, a simple and effective treatment for many post-harvest diseases. Ozone could increase the life of bicarbonate solutions by reducing BOD and clarifying the solution. Ozone also would kill nuisance microbes that contaminate repeatedly used bicarbonate solutions.

Ozone could have a role in reducing fungicide residues in discharge water. More research to assess the benefits on ozone in water should be conducted.

Tests exposing the immobile stage of California Red scale on lemons to ozone in a water bath showed that scale could not be eliminated on the citrus even after a 20-minute exposure. Concentration of the ozone in the bath was measured at 10 parts per million (ppm), the limit for ozone in water at room temperature. Fruit exposed showed no ill effects with the exception that at 20 minutes of exposure, wax in the pores of the skin turned white, indicating some reaction had occurred.

2.3 Ozone as a Gas Fumigant

Facing the need to replace methyl bromide, which is scheduled for final phase-out in 2005, research is being conducted to evaluate the potential benefits of ozone on insects that attack stored agricultural products. For the past 60 years, methyl bromide has been the chemical of choice to protect many agricultural products in post-harvest storage situations for both durable and perishable foods. Methyl bromide has been used for insect control in both quarantine and control situations.

Because methyl bromide has been designated as one of several compounds that contribute to depletion of the earth's ozone layer, its use must be eliminated by 2005. In looking for alternatives to methyl bromide, researchers have suggested that ozone might prove useful under certain situations.

Ozone has the advantage that it can be generated near to the site where it is needed and it is non-persistent in the environment. Ozone readily reverts, after fumigation, to oxygen. Ozone's potential as a replacement for methyl bromide will be enhanced if it can be demonstrated to be effective against some of the more important stored product insects at dose levels that do not cause damage to commodities.

This project report presents data and summary conclusions on the effectiveness of ozone against Indianmeal moth larvae, diapausing codling moth larvae, and California Red scale in simulated storage conditions.

2.3.1 Project Objective

The objective of this project was to evaluate the efficacy of gaseous ozone as a post-harvest fumigant to control selected insects that infest fresh and dried fruit such as codling moth, the Indianmeal moth, the sawtooth grain beetle, the merchant grain beetle and scale insects.

This research task was comprised of two activities. The first activity evaluated ozone as a fumigant in air against insects that commonly attack agricultural produce in storage. The second activity evaluated ozone's effectiveness against a specific scale insect that attacks citrus.

2.3.2 Project Approach

This activity involved the construction of a chamber to facilitate insect exposure to gaseous ozone. A chamber was constructed of high-density polyethylene. On the long side of the chamber, a window made of Lexan was fitted so that it was gas-tight. Several 7 cm holes were drilled in the Lexan, to allow insects to be exposed in 80-mesh monel cages. Silicone stoppers were fastened for insertion into the holes.

Ozone was introduced either from a Del Industries model OZ-151 ozone generator (for low concentrations) or from a Clearwater Tech Inc. model CD10 per AD ozone generator (for high concentrations). Flow of the gas was modified by an air pump that allowed various flows through the corona discharge tube. Humidity control was accomplished by using an adjustable auxiliary air supply that was set to pass air through two jars half full of water

The ozone and supplemental air stream were joined just prior to entering the chamber through a bulkhead union in the lower side of the chamber. Air was allowed to escape the chamber continuously from the top side of the chamber. Mixing of the air in the chamber was accomplished using two 10-cm cooling fans sitting on the bottom of the chamber.

2.3.3 Project Outcome

Results of exposing Indianmeal moth larvae and diapausing codling moths to ozone gas showed that from four to 6 hours were needed for elimination at concentrations of 300 to 500 ppm of ozone. Other insects were not tested because of time constraints, but these exposures and others will continue until an indication of the efficacy is evident.

Because testing of insects was not complete, phytotoxicity and organoleptic effects of ozone gas on various fruits and vegetables phase of the evaluations was not initiated. As soon as a clear picture of the efficacy to insects emerges, testing of several commodities for the effects of ozone on the commodity needs to be completed.

Ozone was shown to be toxic to insect larvae as found by testing against Indianmeal moth larvae and codling moth larvae. The concentrations required for effectiveness, however, are high and the exposure times long. Ozone at these levels, 300 to 500 ppm, is very corrosive due to the oxidizing potential of the gas. Chambers designed for ozone fumigation would need to be made of materials that can withstand the corrosive action of continuous exposure to high concentrations of ozone.

2.3.4 Conclusions and Recommendations

Although work was conducted on two insects that infest commodities, more evaluations need to be performed. To get a clear picture of the efficacy of ozone, more insects and their stages of development need to be evaluated. The results obtained from Indianmeal moth and codling moth diapausing larvae indicate that ozone as a fumigant may be impractical because of the high concentrations and long exposure times required. More data needs to be collected to test this preliminary reservation. Studies need to be conducted on whether or not ozone in its effects on insects follows a concentration x time (CxT) relationship and what that relationship is.

The evaluations of commodity exposure to levels of ozone that kill insects is imperative if recommendations are to be made for its use or non-use. We have fairly good evaluations for fruit in baths saturated with ozone, but this is clearly very different from being exposed to 300 to 500 ppm in air. Some preliminary indications are that not many commodities will tolerate these high levels of ozone for the times necessary to kill insects. Clearly much more work needs to be done in this area. When we have a good idea about the levels and times of exposure to ozone, then commodities can be exposed.

It is too early in the research to make a final recommendation as to whether or not ozone can be used as a replacement for methyl bromide in the treatment of commodities for insect pests. The high concentrations required and the times of exposure for ozone against Indianmeal moth and codling moth larvae would seem to indicate that chambers used for such fumigation will require special materials for construction because of the corrosive nature of ozone. Costs for such facilities may be prohibitive. It is questionable if many commodities can withstand the exposure to high concentrations of ozone required to kill insects. This area needs further investigation and will receive attention following the tests on insect mortality

With only two stages of two insects being tested, however, it is hard to come to any solid conclusion until more research on other insects and other stages of these insects and the ones already tested as larvae are conducted.

2.4 Alternative Fruit Storage Methods

Dried fruits, nuts, and grains are an important component of California's agricultural economy. These products are routinely stored, somewhere along the distribution chain, for periods ranging from a few weeks to several years. Most of these agricultural products are stored in unrefrigerated warehouses or on-farm storage facilities where they are regularly fumigated to protect them from insect infestation.

Chemical fumigants such as methyl bromide and hydrogen phosphide are currently used to protect commodities when they arrive at a storage facility and on a regularly scheduled basis to control insect re-infestations. The expected loss of methyl bromide, a widely used low-cost fumigant for dried products, will place a special burden on the prune industry to adopt alternative methods of insect control.

Alternative fumigants and insecticides are costly, require careful management, and are frequently the targets of emerging environmental regulation. Refrigeration has been used, to a limited extent, for insect control and quality preservation in other dried fruits and nuts. Temperature control is a viable insect control strategy because the insects of concern are dormant when the ambient temperature regime is less than 55 °F.

The University of California at Davis, in cooperation with the United States Department of Agriculture have shown that temperature control is a viable strategy for limiting insect proliferation. This strategy requires an expensive investment for infrastructure, however, and a continuing outlay to pay for energy used.

A research team, from UCD and the United States Department of Energy's (DOE) Lawrence Berkeley Labs, recently completed a study that suggested that comparable levels of insect control could be achieved with a controlled ventilation system that would be less costly and energy consumptive to own and operate than a comparable mechanical refrigeration systems.

In discussions with a dried fruit processor it was learned that some types of fruit are stored in pallet bins that have been encapsulated with plastic-film liners. Indianmeal moths, the main insect pest in stored prunes, do not easily penetrate plastic film. Tightly sealed liners may be a good way of confining existing infestations and protecting fruit that is free of pests from subsequent infestation. The plastic liners also prevent fruit moisture change during storage.

The expected loss of methyl bromide, a widely used, low-cost fumigant for dried products, will require the California prune industry to develop alternative methods for insect control during product storage. In the absence of a viable alternative, up to 25 percent of the prune and raisin crop could be lost because of insect infestation of stored product. In addition, domestic markets could be closed to California growers, an action that would adversely affect other agricultural products.

The most promising alternatives are to store product, previously treated to eliminate insect infestation, below 55 °F to prevent insect feeding and reproduction. Alternatively, previously treated fruit may be stored in a liner that is impervious to insect penetration.

2.4.1 Project Objective

This project subtask was designed to verify performance of a controlled ventilation (CV) system in controlling dried fruit insects and to compare energy efficiency of CV to mechanical refrigeration. The objectives of this project were to:

- Demonstrate the viability and practicality of a low-cost, temperature controlled storage facility to inhibit insect infestation of stored fruit (Section 2.4.2)
- Test the effectiveness of plastic film bin liners to control insect control in stored prunes and (Section 2.4.3)
- Develop baseline data on Indianmeal moth populations near drying and storage facilities (Section 2.4.4).

2.4.2 Controlled Ventilation/Evaporative Cooling

2.4.2.1 Subproject Approach

The Mariani Company in Marysville, California, agreed to work with us to modify one of their storage rooms for testing during the 1997 to 1998 storage season. The room was already insulated and was well suited to allow installation of a controlled ventilation system. The proposed ventilation system used two-16,000 cfm commercial-grade evaporative coolers mounted on the east wall and gravity operated louvers on the opposing wall. A custom-designed controller activated the fans and water pumps during warm weather and just the fans during cooler conditions. The controller was designed to minimize fruit temperature, prevent excessive fruit moisture gain, and minimize electricity use. The first step in the installation process was to request bids for equipment installation.

Bids for installing the evaporative cooling system were requested from two contractors. They were asked to bid on installing two commercial-grade coolers providing air at the top of the east wall and air was exhausted through three gravity-operated louvers, evenly spaced on the west wall. Units were to be controlled with a unit that could select air flow alone if the outside air were cool enough, add water for evaporative cooling at higher temperatures and reduce fan speed for operation in very cool winter months. The least expensive bid indicated a total installed cost of $42,800 (Table 10). The estimate was more than double the projected cost. All of the costs were higher than expected, but louver installation, structural support for the coolers, and electricity and plumbing were particularly high.

Table 10. Cost Estimate – Evaporative Cooling System

Evaporative Cooler Facility Estimate	
Louvers	$7,900.00
Evaporative coolers	$5,200.00
Structural support for the evaporative coolers	$5,000.00
Electricity and plumbing	$9,000.00
Control system	$9,400.00
Air distribution baffles for the coolers	$5,000.00
Other	$1,300.00
Total	$42,000.00

The controlled ventilation design required the installation of many individual components: three louvers, two coolers, and a sophisticated stand-alone controller. Possible redesign was a consideration, with a risk of poorer performance, but the cost would have remained prohibitive for implementation.

Based on rough initial estimates, it appeared possible to install a mechanical refrigeration system for nearly this same price. Refrigeration would cost more to operate than evaporative cooling, especially in the summer months, but would guarantee insect control under all weather conditions and throughout the year.

Based upon this determination, it was decided to request bids for installing 21 tons of mechanical refrigeration in the same storage facility. A commercial grade direct expansion system was proposed with a reheat section on the evaporator coil to provide storage conditions of 55 °F and 60 percent relative humidity. Two of three companies returned acceptable bids and both were close to $52,000. While this exceeded the project budget, it proved that installation of mechanical refrigeration was only about 25 percent more expensive than evaporative cooling. Mechanical refrigeration, however, would have the advantage of providing year-round insect control.

2.4.2.2 Subproject Outcome

Based on this bidding process and other work done on alternatives to methyl bromide, performance and cost comparisons of the options for controlling insects in dried fruit storage was developed (Table 11).

Table 11. Cost of Alternative Insect Control System

	Typical Period Below 55 °F	Effective Control Period (days)	Operating Cost ($ per year)	Capital Cost ($)	Annual Cost[5] ($)
Existing system MeBr + Vapona[1]	15 Dec to 15 Mar	365	$3,500 for fumigant, labor and insecticide[1]	0	$3,500
Ventilation only	15 Nov to 20 Apr	160	$1,200[2]	$8,000	$2,200
Evaporative cooling	1 Oct to 1 May	210	$2,000[2]	$42,800[4.00]	$8,400
Refrigeration	All year	365	$6,660 for electricity	$52,000[4.00]	$14,300
Plastic film bin liners	—	Under test	$5,000 for bags, tape and labor[3]	0	$5,000

[1] Based on 1990 California Prune Advisory Board funded study with updated estimates of MeBr cost

[2] Twice the cost reported in 1997 California Institute for Energy Efficiency draft report by Boghosian, Rumsey, Hakim, Thompson

[3] Bags cost $1 each for 2,700 bins

[4] Based on 1998 bids

[5] Operating cost plus capital costs amortized over 10 years, 8 percent interest, no tax effects considered

Mechanical refrigeration was shown to be the only one of the alternatives examined, by this research task, that is proven to provide year around control. The bin liners may work but additional testing is needed to confirm their efficacy. Adding fan ventilation to an existing storage allows temperature to be maintained below the control threshold for 160 days in a typical year. This compares with about 100 days in the existing storage design. Adding evaporative cooling extends the low temperature period to about 210 days. During this time about 60 to 70 percent of the fruit would have been processed and packaged, so it would protect a great deal of the crop but by no means all of it. Summer storage would be a special concern in years of high production where a great deal of fruit is held over for processing in the following season.

The annual cost data shows that the evaporative cooling system costs $8,400 per year, equal to $3.30 per ton of storage capacity. Mechanical refrigeration costs $14,300 per year, $5.30 per ton of storage capacity. The additional $2 per ton allows year around protection. About 80 percent of the electricity costs are for operation in June through September, the warmest months of the year. Considering the relatively high cost of evaporative cooling and that it provides protection for only seven months a year, we believe that most processors would opt to invest in mechanical refrigeration.

2.4.3 Bin Liner Storage

2.4.3.1 Subproject Approach

Prunes were received on October 13, 1997 and bagged shortly after. The filled plastic bins were weighed shortly after prunes were bagged. Bins were stored in a traditional storage that was fumigated near the end of November. Bins were weighed and then opened on October 8, 1998.

Samples were taken to hold for moth emergence, and Mariani quality control ran a quick quality check on samples from each bin. Samples were also analyzed for quality by the DFA laboratory in Fresno, California.

A second bag storage test was begun November 30, 1998 and ended June 9, 1999. Five bins with bagged prunes were again stored inside with traditionally stored fruit. Fruit was not fumigated prior to bagging. The storage was fumigated once in the fall and again in the late spring. During the same storage period, another set of five bins with sealed bin liners was stored under a roofed building away from the fumigated storage. The liners were sealed by twisting the excess bag top and then tapping the twisted end down against the bag surface in contact with the top of the fruit in the bin. Prune quality was observed and we searched the fruit for signs of insect activity at the end of the test.

Plastic bags of three different materials were tested: 1.2 mil polypropylene, 1.5 mil polyethylene, and 3.0 mil polyethylene (the latter were standard zip-lock bags with the locking end cut off). All bags were 10 inches x 10 inches and sealed on three sides. Prunes were held at 50 °F for several weeks to kill all insect eggs. About 1 quart of prunes was added to each bag, with about 250 g of moth rearing medium (wheat bran based). The bags were sealed by folding the tops over three times, taping the folded top to the bag, and then folding and taping the top one more time. Three bags of each material were placed in each of three 30 liter battery jars, for a total of nine treatment bags for each material. A control bag of each material was also placed in each jar. Controls were identical to the treatment bags, except a 2 inch slit was cut into the top of each bag, allowing easy access to the interior. The jars were tipped on their sides, and the bags were randomly placed in a row within the jars, with the folded tops of the bags upright. Indianmeal moth eggs (0 to 48 hours old) were placed on the sides of the bags at a rate of about 60 eggs per bag. The jars were held at room temperature (about 75 to 80 °F) for 1 week, then the contents of each bag moved to a two quart plastic container and held at 75 °F for emergence of adult moths.

2.4.3.2 Subproject Outcome

Results of the 1998/99 test determined prune quality to be excellent after 1 year of storage in plastic bags. No live or dead insects were recovered from any samples taken. All insect damage detected in the dried fruit was apparently done to the fresh prunes before drying. The lined bins had been left in the storage facility during fumigation. Therefore, it is not certain that the excellent level of insect control observed was solely attributable to the bin liners.

Moisture content did not change during storage. Representatives from the project's industry partners volunteered that the fruit was exceptionally free of sugaring and mold compared to fruit stored conventionally.

During the second season the prune quality was good in the bins stored inside a conventional storage facility (Table 12). The fruit expressed some sugaring at a level that was similar to that observed for fruit that had been stored conventionally.

Table 12. Quality of Prunes Stored in 3 Mil Polyethylene Bin Liners, 1998/99 Storage Season

Bin	Condition	Weight Before (lbs.)	Weight After (lbs.)	Defect After (%)	Moisture After (%)	DFA Damage (%)	DFA Count
1	Field Run	794	793 (-1)	9 (no mold)	18.0	4%	81
2	Field Run	855	853 (-2)	7 (no mold)	19.5	4%	82
3	57/61	521	520 (-1)	12 (no mold)	17.0	3%	57
4	82/86	523	523 (-0)	11 (no mold)	18.5	6%	86
5	42/46	566	566 (-0)	18 (no mold)	19.0	6%	51

The bins used for this test were stored outside and subjected to normal insect pressures. Adult Indianmeal moths were readily observed, with just a casual inspection, in four of the five bins.

The fruit used in this test had been at the dehydrator for several weeks prior to storage and may have been infested during this period. It is also possible that insects may have penetrated the plastic film or entered through the folds in the twisted end of the bin seal.

Mold developed on the top layer of fruit in the top three bins of a five-bin stack. The mold formation is attributable to diurnal temperature swings that cause condensation to form on the inside surface of the liners. It appears that bins with liners must be stored in an environment that prevents significant diurnal temperature fluctuations.

None of the materials were capable of completely protecting the prunes from infestation by Indianmeal moth (Table 13). One-third of both the polypropylene 12 and polyethylene 15 bags were infested, although the infested polyethylene 15 bags produced far fewer moths. Forty-four (44.4) of the polyethylene .003 bags were infested, the infested bags produced more moths than the polyethylene 15 bags, but only a third of the moths found in the polypropylene 12 bags.

Table 13. Results of Laboratory Tests with Bagged Prunes

Jar	Rep	1.2 Mil Polypropylene	1.5 Mil Polypropylene	3 Mil Polypropylene
A	1	0	0	1
	2	0	2	0
	3	25	0	0
	Control	52	51	29
B	1	0	1	0
	2	0	0	0
	3	0	0	23
	Control	52	60	26
C	1	20	0	10
	2	42	2	0
	3	0	0	8
	Control	59	62	17
Percent infested		33.3%	33.3%	44.4%
Average moth count		29.0	1.7	10.5
Average moths in controls		54.3	57.7	24.0

The control bags of polyethylene .003 produced less than half the number of moths as the other two materials. One possible explanation for this is that the neonate Indianmeal moth larvae may have more difficulty in climbing up this material to reach the opening.

Because we were unable to find obvious signs of direct penetration by the neonate larvae through the bag material, it is possible that the failure of the bags was due to poor sealing of the opening. Neonate Indianmeal moth larvae are capable of taking advantage of very small cracks and gaps in package seals. Slightly thicker material and more effective seals may be needed to provide suitable protection in storage.

2.4.4 Insect Monitoring

2.4.4.1 Subproject Approach

Management strategies developed by the project must be evaluated through some type of insect sampling program. Insect infestation levels in commercial prune processing plants are very low, which makes evaluation through direct product sampling difficult. Indirect trapping methods using pheromones are the easiest to implement, but do not give direct measures of product infestation. We chose to use both pheromone traps and prune samples to develop base line population and product infestation data. We also attempted to develop a mechanical method for sampling entire prune bins.

Pheromone traps: Wing traps baited with Indianmeal moth pheromone lures were placed at the Mariani prune plant on October 9, 1997. Two traps were placed within each half of the prune storage area, and additional traps were placed outside the two main entrances, for a total of six traps. Traps were checked for adult moths every 7 to 14 days. Pheromone lures were replaced every 6 to 7 weeks. Additional traps were placed at an outlying prune dehydrator.

Prune samples: Ten 1.5 l prune samples were taken randomly from the bottom bins of each warehouse area (south and north). Each sample was mixed with 100 ml of wheat bran diet and placed in 4-liter (L) plastic containers. The containers were held at 25 °C for 6 weeks. Any emerging adult moths were recovered and identified. An additional prune samples were also taken from an outlying prune dehydrator.

Mechanical sampling: A portable conveyor belt was used to propel prunes infested with Indianmeal moth larvae against a metal plate coated with honey. Any larvae found on the plate were counted. The prunes were then carefully examined for additional larvae.

2.4.4.2 Subproject Outcome

Pheromone traps: Three species of pyralid moths were recovered in the pheromone traps placed at Mariani; Indianmeal moth (*Plodia interpunctella*), raisin moth (*Cadra figulilella*) and the Mediterranean flour moth (*Ephestia kuehniella*). Overall, Indianmeal moth was the species most often collected (92 percent) followed by raisin moth (6.5 percent) and then Mediterranean flour moth (1.5 percent) (Table 14). Mediterranean flour moth was found only in the two outside traps. Nearly 97 percent of the raisin moth collected were from the outside traps. Indianmeal moth was the predominate species recovered in the warehouse area (99.8 percent) but only 16.7 percent of the moths captured on the outside traps were Indianmeal moth. This indicates that Indianmeal moth is the species of most concern within prune storage.

Table 14. Summary of Pheromone Trap Results for Mariani Prune Processing Plant

Location	Indianmeal Moth	Raisin Moth	Mediterranean Flour Moth	Total Moths
Warehouse	3,798	9	0	3,807
Outside	66	266	64	396
Total	3,864	275	64	4,203

Within the warehouse, Indianmeal moth captures were highest in October, 1997. Moth numbers dropped off quickly in November, after the facility was fumigated and Indianmeal moths entered diapause. No Indianmeal moths were caught from mid-November until mid-May. Moth numbers increased more rapidly in the North warehouse than in the South warehouse, but levels in neither warehouse reached those of the first year. Very few Indianmeal moths were caught in the spring and summer of 1999. This was largely due to the initiation of a weekly pyrethrin fogging program in late May.

Indianmeal moth counts were elevated during the period, mid August through mid September, until the fruit was transported to storage in November. This indicates that the fruit, which is completely disinfested during drying, is under significant reinfestation pressure while it is stored at the drying facility. The liner storage system will work well only if the fruit is virtually free of infestation before being placed in the liner. This may require that:

- The fruit is placed in the liner immediately after drying
- It is protected from infestation between drying and bag storage
- It is disinfested again just as it is placed in the bag.

Raisin moths made up a larger percentage of the total moths captured during the fall of 1997. This may be due to the presence of prunes of higher moisture content that would be more susceptible to infestation by raisin moth. Traps were not monitored after prunes had been removed to the processor (early November). We began monitoring again after Indianmeal moth was recovered at the prune processing plant (late June). Although raisin moth was recovered again during the summer months, no Indianmeal moths were recovered. It is likely that the raisin moth being collected in the summer months is from the surrounding orchards.

Prune Samples (Table 15). Neither Indianmeal moth nor raisin moth were recovered at high levels from samples taken from the prune processing plant. Raisin moth was found in only 2 out of 20 samples taken on one sample date.

Table 15. Percentage of Samples Infested With Indianmeal Moth or Raisin Moth

	Processing Plant		Remote Dehydrator	
Date (1998)	Indianmeal Moth	Raisin Moth	Indianmeal Moth	Raisin Moth
June 11	5	10	—	—
September 3	15	0	—	—
September 16	15	0	20	0
October 1	0	0	10	0
October 15	10	0	30	10
October 26	5	0	10	30
November 11	45	0	40	20
December 3	0	0	—	—

With the exception of one sample date, Indianmeal moth was usually found in no more than three samples (15 percent). On November 19, Indianmeal moth was recovered from 45 percent of the samples taken, despite the fact that samples taken two weeks before and after showed low levels (five percent) or no Indianmeal moth present. Raisin moth was recovered more often from samples taken from the remote dehydrator. Indianmeal moth was also recovered more consistently from remote hydrator samples than from samples taken from the processing plant.

Holding prune samples for Indianmeal moth emergence was a more sensitive method for detecting infestations than casual examination of the prunes, however, it was time consuming and required considerable space for the samples. The erratic results also indicate that the population levels found within the prunes are quite low and unevenly distributed. Increasing the number of samples taken would increase the accuracy of the method, but would require resources unavailable to us at the present time.

Mechanical sampling: Preliminary studies with the portable conveyor belt indicated that only a very low percentage of larvae were recovered. The percentage of larvae recovered was also not consistent. We decided that mechanical sampling would not be effective and that further tests were not necessary.

2.4.5 Conclusions and Recommendations

We determined that, contrary to previous research, a storage temperature control system for dried prunes that uses controlled ventilation/evaporative cooling is only about 20 percent less expensive to install than mechanical refrigeration. It also could not provide temperature control below 55 °F in the summer months. The controlled ventilation/evaporative cooling system was too expensive to install, considering that it would provide storage temperatures for safe storage (below 55 °F) for only 7 months a year. Evaporative cooling is not effective for the 5 months of the year because of the hot weather during which maintaining-cooling below 55 °F may not be possible. Packers will most likely choose to spend more for mechanical refrigeration and gain the benefit of year-round insect protection. Refrigeration will be more expensive than methyl bromide fumigation but is not a high cost considering the value of the fruit.

For these reasons we believe that prune processors will install mechanical refrigeration for insect control and therefore we did not install to proposed demonstration system.

The liner storage system was only partially successful as a storage system. It allowed good preservation of fruit quality but it has not yet been developed as a dependable method of protecting prunes from insect attack.

Based on field and laboratory tests we concluded that storage of prunes in plastic-film bin liners appears to produce good quality fruit and provides a low cost method of protecting stored fruit from reinfestation. In field and lab tests, however, this strategy was not always effective in excluding Indianmeal moth. It is possible that complete protection against Indianmeal moth infestation may be accomplished by the use of better bag sealing methods. Future research will be done to improve bag storage methods.

2.5 Dairy Wastewater Management

California leads the nation in the production of eggs and milk. The active laying chicken flock numbers about 25 million birds and the combined dairy herd includes approximately 1.2 million dairy cows. In addition, the state is a major producer of poultry, beef, and sheep.

Most of the dairy, poultry, and swine operations in the state are concentrated in the San Joaquin Valley and southern California. Favorable economies of scale, however, combined with escalating land values, are driving concentration of the State's livestock onto a dwindling acreage of farmland even as the flocks and herds are increasing in size.

Up to 40 percent of the dry matter and from 75 to 85 percent of the nutrients fed to livestock is excreted in the manure and the subsequent deposition of manure in holding ponds or on spreading grounds is contributing to a serious deterioration of water quality throughout the State. Moreover, odors and gases produced by decomposition of manure in storage are a primary concern of air quality regulation.

Animal operations are considered to be the major agricultural source for ammonia emission in California. The harmful effects of ammonia emission in California is of concern because of its contribution to the formation of ammonium nitrate particles which contribute to degraded air quality; particularly during the fall and winter.

Manure management is an important task for every livestock operation. Manure in dairy, layer, and swine operations is commonly handled as liquid or slurry manure by means of a hydraulic flushing—lagoon storage—irrigation system.

The management of nutrients (nitrogen and phosphorus) from livestock manure is vital for protection of the state's water resources. The Central Valley Regional Water Control Board (RWQCB) enacted a General Waste Discharge Requirement (WDR) on October 26, 1996 which established limits for the discharge of nitrogen and phosphorus. The WDR immediately impacted 110 Central Valley dairy operations, which needed to implement nutrient management plans for handling manure.

The project activity associated with this task involved laboratory and field trial testing of an aerobic treatment system that will assist dairy herd operators to comply with WDR requirements.

The goal of this project was to develop and demonstrate an energy efficient, integrated wastewater management system for California's livestock operations. The successful system would enable water recycling and reuse, improve animal herd health and food safety, and reduce pollutant discharge.

Aeration is the key wastewater treatment component in the wastewater treatment system evaluated. The research objective in the first year was to develop an effective aerobic treatment process that could significantly reduce energy consumption (compared to conventional treatment processes) while still meeting the regulatory requirements for effluent quality on dairy farms.

Manure management requires a significant amount of labor and capital investment for collection and disposal systems. Manure in dairy, layer (chicken), and swine operations is commonly handled as liquid or slurry (by means of a hydraulic flushing) and stored in lagoons for subsequent land application by irrigation.

The major problems associated with manure management on livestock farms are high solids and nutrient contents of the wastewater. High solids content causes fast sludge buildup in storage lagoons, thus reducing the available storage volume, and high solids loading to the crop land following irrigation application which hinders crop seed germination and growth. High nutrient contents tend to cause overloading of land with nutrients, especially nitrogen, which leads to contamination of water resources.

Odors and gases produced during the decomposition of manure in storage are primary air quality concerns. Animal operations are considered to be the major agricultural source for ammonia emission in California. It is believed that about 46 tons per day (60 percent of the total) of the particulate emissions in the southern San Joaquin Valley can be attributed to animal operations. The harmful effects of ammonia emission and deposition on the vegetation in the vicinity of animal operations are well known.

In response to President Clinton's Clean Water Action Plan (CWAP), the USDA-EPA issued Unified National Strategy for Animal Feeding on March, 1999. This plan calls for concerted efforts for livestock producers to make environmentally sound animal manure management plans and minimize the water quality and public health impacts of animal feeding operations. The concerns embodied within this report have driven the search for alternative wastewater treatment methods for livestock producers.

2.5.1 Project Objective

The objective of this project was to examine the functionality of an innovative biological treatment device to manage the disposal of liquefied animal wastes. Aerobic treatment is a biological treatment process that uses bacteria to degrade organic wastes in the presence of oxygen. Aerators are electric-driven devices that are used to supply oxygen to the bacteria in the wastewater. The amount of electrical energy required for aeration is directly related to the amount of oxygen needed to oxidize the wastewater chemical elements, such as carbon and nitrogen.

Aeration is generally considered to be a reliable technology for wastewater treatment and has been widely used for the treatment of municipal and industrial wastewater. Aerobic treatment has previously been explored as a treatment option for animal wastewater by a number of researchers. Activated sludge, oxidation ditch, and extended aeration are widely used aerobic treatment processes. Due to high-energy consumption used by these systems to supply oxygen (air), however, aerobic treatment has never evolved as a practical option for livestock producers (Westerman and Zhang, 1997).

Animal wastewater normally has much higher (>100 times) organic strength and nutrient content than normal municipal wastewater. The engineering design and operational guidelines of aerobic treatment systems developed for municipal wastewater treatment are not directly transferable to animal wastewater treatment. In addition, the objectives for animal wastewater treatment are different from municipal wastewater treatment.

2.5.2 Project Approach

This project studied the sequencing batch reactor (SBR) as the technology of choice because of its distinct relationship to conventional aerobic treatment processes. The SBR treats wastewater in small batches and fits well with most animal wastewater collection systems. It is a time-oriented system, operates over repeated cycles of five phases—fill, react, settle, decant, and idle—and allows longer solids retention times than hydraulic settling retention time. The settling phase in the treatment system after bacterial reactions enhances the flocculation of solids in the wastewater and makes the solid-liquid separation possible by gravity settling, thus allowing collection of solids in a separate stream and generation of recyclable water for farm uses.

The major factors that control the performance of SBRs include organic loading rate, hydraulic retention time (HRT), solids retention time (SRT), dissolved oxygen (DO), and influent characteristics such as solids content and carbon to nitrogen ratio (C/N). Depending on how these parameters are controlled, the SBR can be designed to have one or more of these functions—carbon oxidation, nitrification and denitrification.

Carbon oxidation and denitrification are carried out by heterotrophic bacteria and nitrification is by autotrophic bacteria. SBR has been successfully used for wastewater treatment applications in small communities. The SBR is a relatively new technology for agricultural applications. Previous research on the SBR for animal waste was primarily concentrated on swine wastewater treatment.

Previous research findings about the SBR for treatment of swine manure and other types of wastewater provide valuable references for the treatment of dairy wastewater. Due to the differences in the characteristics of dairy wastewater from other types of wastewater, however, research is needed to develop design and operational guidelines for the SBR in treating dairy wastewater of various characteristics. All previous research was focused on single-stage SBR where carbon and nitrogen oxidation occurs in a single reactor. This type of operation usually exerts high oxygen demand, therefore energy demand, especially for animal wastewater, which has high BOD/N ratio

In addition to the single-stage SBR system, this project also explored the concept of two-stage SBR operation, which separates carbon oxidation and nitrogen oxidation in separate reactors to optimize the growth environment for different bacteria and thereby minimize the energy requirement. One-stage and two-stage SBR systems were compared in the laboratory study of dairy wastewater treatment. The effects of various factors, such as HRT, SRT, organic loading rate, and inhibition on the performance of the SBR in treating dairy wastewater for solids and nitrogen reduction were determined.

This research was carried out in both laboratory- and pilot-scale. The research procedures for the laboratory and field research are described in the following.

2.5.2.1 Laboratory Research

Aerobic treatment of dairy wastewater with sequencing batch reactors was evaluated in the laboratory under different operating conditions using automated bench-scale bioreactors. Both single-stage and two-stage treatment systems were tested.

The single-stage SBR system consisted of an SBR and a solids-settling tank in series. The wastewater was first fed into the SBR for treatment and the effluent of the SBR including both sludge and liquid was then discharged into a settling tank, where the liquid was separated from sludge by gravity settling and characterized as liquid effluent of the system.

The two-stage system (Figure 21) consisted of an SBR (first-stage reactor), a solids-settling tank and an SBR (second-stage reactor) connected in series. The SBR and solids-settling tank were operated in the same way as described above. The liquid effluent obtained from the solids-settling tank was further treated in the second-stage mainly for nitrification.

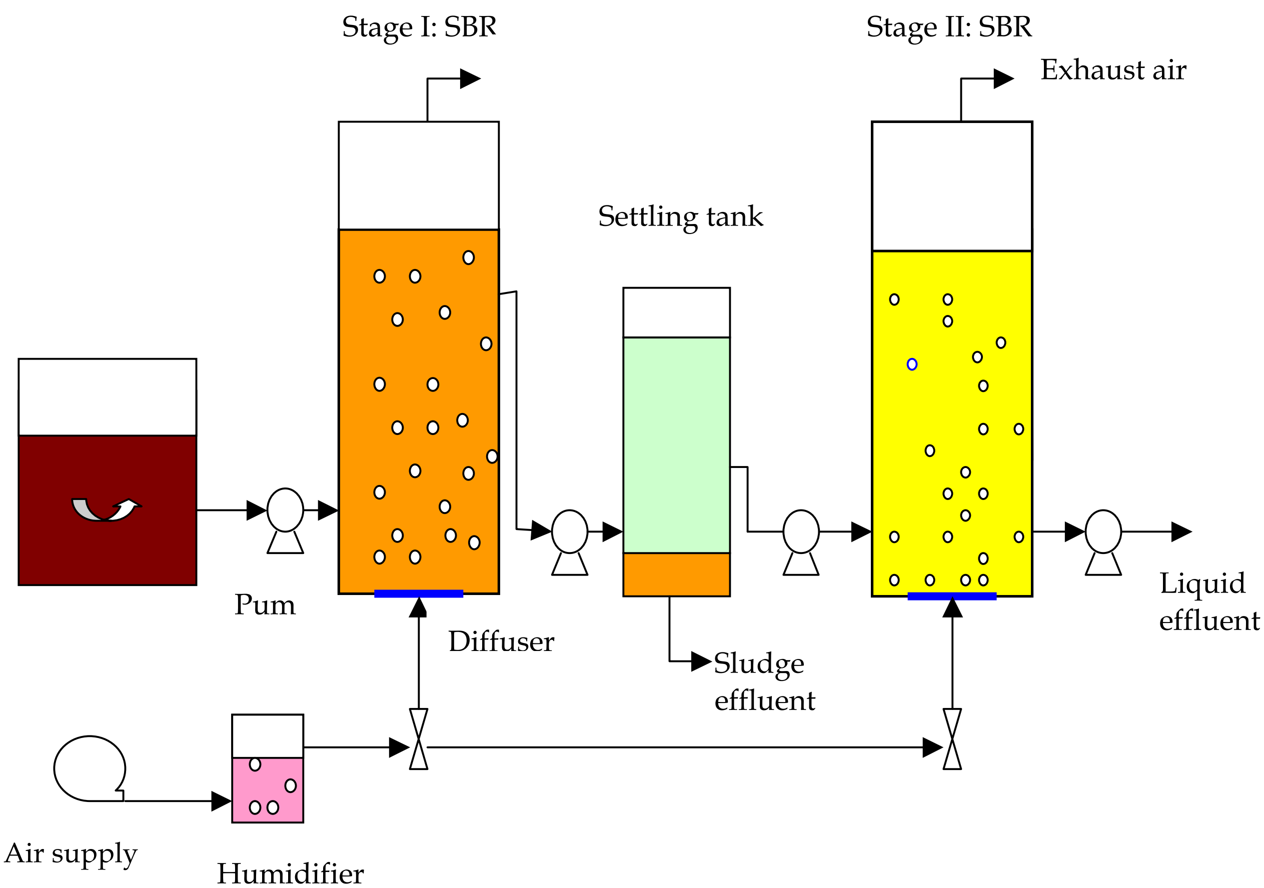


Figure 21. Two-Stage Laboratory System

Each system was fed and decanted twice a day with 12 hours in each treatment cycle. All the peristaltic pumps used for feeding and decanting were operated automatically with a digital time controller. The time sequence for different actions during each treatment cycle of the SBR was one to three minutes fill, 11-hours mixing, and four to eight minutes react, 40 minutes settle, one to three minutes decant, and ten minutes idle. The amount of solids wasted in the effluent from the SBR each day was controlled to provide the desired SRT.

The SBR reactors were made from transparent acrylic and had a total volume of 6-L volume in each with 51-cm in height and 12-cm in diameter. During testing, the liquid volume of each reactor was three L. Each reactor was aerated using pressurized air at a controlled flow-rate. To minimize the water evaporation in the reactor, the air was humidified by traveling through water contained in a 15-L jar prior to entering the reactor. The air was evenly distributed into the wastewater through four air stone diffusers installed near the bottom of the reactor.

The reactors were initially seeded with the activated sludge obtained from the UC Davis Wastewater Treatment Plant and allowed to acclimate for about two months before formal experiments were started. It normally took about four weeks for each SBR reactor to reach a steady state when a new operating condition was introduced. The steady state was defined to be a state when the weekly variation of effluent COD, TS, NH_3-N, and pH were less than 5.0. These parameters were monitored twice a week. To determine the ammonia emission from SBR due to aeration, ammonia in the exiting air of SBR was collected by absorbing it with 0.3 N boric acid solution for 24 hours under each testing condition.

Dairy manure was collected on the Dairy Research Farm of the University of California at Davis. Due to runoff of urine on the feedlot, the collected manure was mainly feces and contained a relative low content of ammonia nitrogen. The manure was slurried by the addition of water and then screened twice with two sieves with openings of 4x4 mm and 2x2 mm, respectively, to remove large particles. The screened manure was transported immediately to the laboratory and stored in a freezer at -20 °C until use.

The total solids (TS) and COD of the screened manure were 30,000 and 40,000 mg/L and 35,000-50,000 mg/L, respectively. When needed, the stored manure was thawed and then diluted with tap water to obtain a desired COD concentration. Due to the relatively low ammonia content of the raw manure, as compared to typical levels in the manure collected on dairy farms, urea was added to increase the NH_3-N in the prepared manure from 100-125 mg/L to 500-550 mg/L. The prepared manure was then put into a 50-L feeding tank housed in a refrigerator at 4 °C for daily use. The feeding tank had an agitator to mix the wastewater during the feeding of the reactors.

The experiment was carried out in two phases. The first phase was for studying the effects of operating parameters on the performance of the single-stage SBR system. The parameters investigated included DO, HRT, and organic loading rate. The second phase was to evaluate the performance of a two-stage SBR system. The two systems were compared in terms of solids removal and nitrogen conversion efficiencies.

With the single-stage system, the effect of DO was first examined by testing the SBR at two DO levels, 2.5 and 4.5 mg/L, using the wastewater with 10,000 mg/L COD. From the results, it was found that there was no significant difference between the two DO levels in terms of effluent quality and the 2.5 mg/L DO was considered to be sufficient for bacterial growth. Therefore, 2.5 mg/L DO was used for later test runs.

The effects of HRT and COD loading rate were investigated. Using the wastewater with 10,000 mg/L COD, three HRTs (1, 2 and 3 day) were tested. The SRT of the SBR was controlled at 15 days for all three HRTs. The SRT was controlled by discharging a proper amount of mixed liquor in the reactor prior to decanting the supernatant. Based on the results with 10,000 mg/L influent COD, two HRTs (1 and 3 days) were then selected for further testing with the wastewater of 20,000 mg/L COD. Due to fast sludge build-up in the reactor at the higher loading rate, the SRT of the SBR was decreased to 1.5 days and 4 days for 1-day and 3-day HRT, respectively.

The performances of the treatment systems were evaluated in terms of liquid effluent quality and solids and nitrogen removal efficiencies. The liquid effluent quality was measured by the remaining concentrations of TS, volatile solids (VS), COD, soluble COD, TKN (Total Kjeldahl Nitrogen), NH_3-N, NO_2-N and NO_3-N in the liquid effluent. Two kinds of removal efficiencies were used to interpret the results for solids and nitrogen removals. One efficiency, E_t, is based on the removal from total effluent (including both sludge and liquid effluent generated). The other efficiency, E_l, was based on the removal from the liquid effluent.

For the single-stage SBR system, the total effluent was the effluent from the SBR and the liquid effluent was the supernatant decanted from the solids settling tank. For the two-stage SBR system, the total effluent was the combination of sludge from the settling tank and the liquid effluent from the second-stage SBR and the liquid effluent was the effluent of the second-stage SBR. Most of previous research reports only included removal efficiency from liquid effluent (E_l). Actually, to assess the system efficiency for reduction of solids and nutrients in the wastewater, the removal efficiency from total effluent (E_t) is an important parameter because sludge effluent needs attention in terms of waste management.

After each reactor reached steady-state under testing conditions, samples were taken from the influent, mixed liquor, total effluent, and liquid effluent of the reactor three times a week (every other day) for COD, soluble COD, TS, VS, NH_3-N, NO_2-N, NO_3-N, and TKN analyses. The removal efficiencies, E_l and E_t, were calculated based on the data from influent, liquid effluent and total effluent the systems. The accumulation rate of each constituent was defined by determining the difference in its concentration in the mixed liquor of the reactor between 2 consecutive days and was included in the mass balance calculations.

The separation of sludge and liquid in the total effluent of the SBR was performed by settling the effluent in a 1-L graduated cylinder for 10 hours and then decanting the liquid fraction above the sludge-liquid interface line. The sludge volume was recorded and calculated as a fraction (percentage) of total volume (1-L). The sludge volume fraction is used as the indicator for sludge settleability. The COD, soluble COD, TS, VS, and TKN were measured according to the standard methods (APHA, 1995). The pH was measured with an Accumet pH meter. The NH_3-N was measured with a gas-sensing electrode and the pH meter. The DO in the reactors was monitored on daily basis with a DO meter (YSI Mode158). The NO_2-N was analyzed with the HACH method using DR/2000 spectrophotometer. The NO_3-N was measured with a diffusion-conductivity analyzer (Carlson, 1978).

2.5.2.2 Field Research

A pilot two-stage SBR treatment system was evaluated on a dairy farm in Hanford (Figure 22). The farm has 800 dairy cows and uses hydraulic flushing to collect manure from free-stall feeding barns. The daily wastewater generation on the farm is about 16,000 gallons per day.

Figure 22. Pilot-Scale Two-Stage Sequencing Batch Reactor System

The wastewater flows first through a solids-settling basin to allow heavy and large particles to settle out before the wastewater enters an anaerobic lagoon for storage. Solids build-up in the wastewater storage lagoon and high nitrogen content are the primary concerns of the dairy farmers. Two sequencing batch reactors with 10,000-gallon volume in each reactor were set up between the solids-settling basin and the anaerobic lagoon.

From October 15, 1998 to March 15, 1999, the first-stage SBR was evaluated as the single-stage treatment with a three-day hydraulic retention time. The SBR system was used to treat part of the effluent from the solids-settling basin. On March 16, 1999, the second-stage reactor was brought on line to treat the effluent from the first-stage reactor and the two reactors were operated in series for three weeks until April 6, 1999. Each reactor was aerated using a venturi air-injector with the dissolved oxygen concentration controlled at above two mg/L.

The pilot SBR system was extensively monitored by taking samples from five locations in the treatment system three times a week for analysis of TS, VS, COD, TKN, NH_3-N, NO_3-N, and NO_2-N. The reductions of these parameters were determined based on the mass balances. The temperature of each SBR reactor was recorded continuously over the period of testing.

2.5.3 Project Outcome

2.5.3.1 Laboratory Research Results

Table 16 and Table 17 show the effluent quality and treatment efficiencies of the single-stage SBR system for treating the dairy wastewater with 10,000 mg/L and 20,000 mg/L COD respectively. For the same HRT, increasing the influent COD would increase the COD loading rate of the treatment system. For the same influent COD, decreasing the HRT would also increase the organic loading rate. The soluble COD in the influent was 29 to 33 percent of total COD and the VS was 77 to 81 percent of TS. The effects of HRT, SRT, and organic loading rate on the performance of the treatment system in terms of COD, solids and nitrogen removals from the wastewater are presented as follows.

Table 16. Effluent Quality and Treatment Efficiencies of SBR for 10,000 mg/L Influent COD

		1-Day HRT				2-Day HRT				3-Day HRT			
Para-meters	Influent (mg/L)	Liquid Effluent (mg/L)	Sludge Effluent (mg/L)	E_l (%)	E_t (%)	Liquid Effluent (mg/L)	Sludge Effluent (mg/L)	E_l (%)	E_t (%)	Liquid Effluent (mg/L)	Sludge Effluent (mg/L)	E_l (%)	E_t (%)
COD	10,000	1,980	60,667	80.2	45.0	1,580	65,000	84.2	48.7	1,470	70,800	85.3	50.7
SCOD	2,914	1,457	1,457	50.0	50.0	1,451	1,451	50.2	50.2	1,428	1,428	51.0	51.0
TS	6,656	2,436	49,033	63.4	21.4	2,476	49,969	62.8	23.7	2,416	56,044	63.7	23.4
VS	5,108	1,724	29,030	66.2	34.2	1,532	32,199	70.0	36.4	1,400	33,713	72.6	41.0
TKN	780	195	3,029	75	53.2	185	3,204	76.3	54.6	165	3,619	78.8	56.7
TN	780	481	2,574	38.3	22.2	480	2,577	38.5	23.4	490	2,605	37.2	23.6
NH_3-N	510	120	120	76.5	76.5	105	105	79.4	79.4	70	70	86.3	86.3
NO_3-N	0	37				55				45			
NO_2-N	0	249				240				280			
pH	8.1	6.8				6.7				6.7			
Volume Fraction (%)		94	6			94.4	5.6			95	5		

Table 17. Effluent Quality and Treatment Efficiencies of SBR for 20,000 mg/L Influent COD

		1-Day HRT				3-Day HRT			
Parameters	Influent (mg/L)	Liquid Effluent (mg/L)	Sludge Effluent (mg/L)	E_l (%)	E_t (%)	Liquid Effluent (mg/L)	Sludge Effluent (mg/L)	E_l (%)	E_t (%)
COD	20,000	4,300	64,375	78.5	30.4	2,660	76,923	86.7	38.4
SCOD	6,660	3,197	3,197	52.0	52.0	2,005	2,005	69.9	69.9
TS	12,442	4,367	48,990	64.9	18.7	3,498	50,246	71.9	23.0
VS	10,104	3,142	41,174	68.9	21.6	2,809	37,618	72.2	27.4
TKN	1,140	540	2,786	52.6	21.1	170	3,473	85.1	47.4
TN	1,140	573	2,822	49.7	18.2	488	3,797	57.2	19.5
NH_3-N	540	310	310	42.6	42.6	80	80	85.2	85.2
NO_3-N	0	20				188			
NO_2-N	0	13				130			
pH	8.0	8.7				7.8			
Volume fraction (%)		84	16			87	13		

COD and Solids Removal From the Wastewater

With 10,000-mg/L influent COD, the SRT of the SBR was controlled at 15 days. As shown in Table 16, with the increase of HRT from 1 to 3 days, the COD, soluble COD, TS, and VS in the liquid effluent became lower, yielding better effluent quality due to increased biological conversion and improved sludge settleability as indicated by the increased removal efficiencies (E_l and E_t) and decreased sludge volume fraction.

The improvement in the liquid effluent quality and removal efficiencies in terms of COD, soluble COD, TS, and VS with the increase of HRT was not significant. For example, the increase of COD removal efficiency in the liquid effluent (E_l) was about five when HRT increased from one to three days. All three reactors were successful in achieving nitrification with similar nitrogen conversion rates. This suggests that 1-day HRT was sufficient for treating the dairy wastewater with 10,000 mg/L COD.

At one-day HRT, the removals from liquid effluent as compared to the influent are 80.2 percent COD, 63.4 percent TS and 66.2 percent VS. These removals were due to biological conversion in the SBR and sludge separation in the solids-settling tank. The removals due to biological conversion alone in the SBR, as measured by E_t, are 45 percent COD, 21.4 percent TS, and 34.2 percent VS. This suggests that the sludge separation after SBR treatment is necessary for achieving significant COD and solids removal from the dairy wastewater.

It was found that aerobic treatment greatly enhanced the flocculation and settleability of the solids in the wastewater as compared to raw wastewater. The COD and VS loading rates of the SBR at 1-day HRT were 10g/L/day and 5g/L/day, respectively. The food to microorganism ratio (F/M), calculated as the ratio of daily COD loading rate to mixed liquor volatile suspended solids (MLVSS) in the reactor, was 1.44.

When the influent COD was increased from 10,000 mg/L to 20,000 mg/L, the SRT of the SBR decreased from 15 days to 1.5 and 4 days for 1-day and 3-day HRT, respectively, due to fast solids build-up in the reactor and poor solids settleability. It was not possible to control the SRT at a higher level. In the 1-day HRT reactor, due to dramatic decrease of SRT to 1.5 days, nitrification vanished quickly due to washout of the nitrification bacteria resulting in very low concentrations of nitrite and nitrate in the effluent as shown in Table 16. At 3-day HRT, however, since the SRT was maintained at 4 days, nitrification was able to sustain in the SBR.

At 1-day HRT and 20,000 mg/L influent COD, the removal efficiencies from liquid effluent (E_l) are 78.5 for COD, 64.9 for TS, and 68.9 for VS, which are close to the efficiencies achieved at 1-day HRT and 10,000 mg/L influent COD. This means that 1-day HRT was good for solids removal from the liquid effluent. If nitrification is desired, longer HRT, such as 3 days, should be used. At three-day HRT, about 59 of NH_3-N or 28 percent of TKN was converted into nitrite and nitrate nitrogen.

The conversion of NH_3-N is similar to the conversion level at 1-day HRT and 10,000-mg/L influent. When the influent COD was increased to 20,000 mg/L, NH_3-N was not proportionally increased to examine the effect of organic nitrogen to ammonia nitrogen ratio on the nitrogen conversion. Based on the TKN removal data for the two levels of COD in the influent, if analyzed from the standpoint of mass balances, it appears that the converted nitrogen was mainly from ammonia nitrogen.

Increase of organic nitrogen in the influent did not cause the increase of nitrification. As compared to the treatment efficiencies at 1-day HRT, the removals of COD and solids at 3-day HRT were three to eight percent higher. Therefore, the difference between one-day and three-day HRTs was mainly on the degree of nitrification.

The sludge separated from the effluent of the SBR contained about 5 TS. The lower influent COD (10,000 mg/L) resulted in better sludge settleability than the higher influent COD (20,000 mg/L). The sludge volume as compared to total effluent volume was 5 to 6 percent and 13 to 16 percent for the lower and higher levels of influent COD, respectively. The sludge is composed of not-degraded solids in the wastewater and newly formed bacterial cells. It can be made into organic soil amendment through dewatering and drying or composting

Nitrogen Conversion in the Wastewater

It was found that the ammonia emission rate from the SBR was small when there was nitrification in the reactor. With 10,000 mg/L influent COD and 1- to 3-day HRT treatment, about 22 to 23 percent of the total nitrogen was lost in the treatment process. The amount of ammonia collected from the exiting air of the SBR during a 24-hour period accounted for only 2 to 3 percent of the total nitrogen. The remaining 20 percent nitrogen lost might be due to emission of nitrous oxides (NO and NO_2), which could be formed during the nitrification processes, and nitrogen gas (N_2) possibly formed in the simultaneous occurrence of nitrification and denitrification processes in the SBR. The nitrous oxides and nitrogen gas in the existing air of the SBR were not measured. With 20,000 mg/L influent COD and 3-day HRT, the loss of total nitrogen was similar to the results above. At 1-day HRT, however, when there was little nitrification in the SBR, the nitrogen loss due to ammonia volatilization was higher, 8 percent of the total nitrogen. About 56 percent NH3-N or 37 percent TKN in the influent was converted into nitrite and nitrate nitrogen. This amount of nitrogen can be potentially denitrified if nitrogen removal from the wastewater is desired.

The variations of NH_3-N, NO_2-N, and NO_3-N in the SBR during a 12-hour operating cycle in treating the wastewater of 10,000 mg/L COD at 3-day HRT are shown in Table 16. Conversion of ammonia mostly occurred in the first 5 hours when NO_2-N increased while NH_3-N decreased. The NO_2-N increased to the peak value after about 5 hours and then started to decrease while NO_3-N started to increase. Due to formation of nitrite and nitrate, pH dropped gradually by about 1.5 unit, from 8 to 6.7, during the 12-hour cycle. The actual variations of NH_3-N, NO_2-N, NO_3-N, and pH in the SBR during the operating cycle depend on the bioconversion dynamics in the reactor, initial ammonia concentration, and alkalinity in the wastewater.

It can be seen that relatively high nitrite concentration was found in the effluent of the SBR and not all the ammonia was converted. This indicates that the nitrification process might be inhibited. It is believed that free ammonia (FA) and free nitrous acids (FNA) are inhibitory to nitrification bacteria above certain concentrations (US EPA, 1993).

Free ammonia (FA) begins to inhibit Nitrosomonas (bacteria responsible for converting ammonia to nitrite) at 10-150 mg/L and Nitrobacteria (bacteria responsible for converting nitrite to nitrate) at 0.1 to 1.0 mg/L. Free nitrous acids (FNA) begins to inhibit Nitrosomonas and Nitrobacteria at 0.22 to 2.8 mg/L. The FA and FNA concentrations in the wastewater are directly correlated to pH and temperature, and concentration, respectively, of total ammonia (ammonia plus ammonium) and total nitrous acids (nitrite and nitrous acids).

The FA and FNA in the SBR tested in this study were in the ranges of 0.07-5.5 mg/L and 0.03-1.05 mg/L, respectively, indicating that there could be slight inhibition of nitrification bacteria, causing partial conversion of ammonia and nitrite in the SBR. Relative high COD to NH_3-N ratio in the influent (19.6 and 39.2 for the two levels of influent COD) can be another reason for the incomplete ammonia conversion due to suppression of nitrification bacteria population by the heterotrophic bacteria. This leads to the need to explore a two-stage treatment configuration where the first-stage reactor is used mainly for carbon oxidation and enhancement of solids settleability and the second-stage reactor is used for nitrification.

After the first-stage SBR treatment and sludge separation, the liquid effluent would have proper carbon to nitrogen ratio (C/N) to support the growth of nitrification bacteria in the second stage. With optimization of environmental conditions and substrate characteristics for heterotrophic and autotrophic bacteria in separate stages, the overall HRT of the treatment system may be reduced as compared to the single-stage system, as indicated from the performance data of a two-stage system presented below.

Performance of Two-Stage SBR System

Table 18 and Table 19 show the performance data of the two-stage system.

Table 18. Performance of Two-Stage SBR-CMBR System for 10,000 mg/L COD Influent

Parameters	Influent (mg/L)	Stage I: SBR (1-Day HRT) Liquid Effluent (mg/L)	Stage II: CMBR (1-Day HRT) Liquid Effluent (mg/L)	E_I (%)	E_t (%)
COD	10,000	1,980	1,374	86.3	51.1
SCOD	2,914	1,457	1,014	65.2	65.2
TS	6,656	2,436	2,076	68.8	24.8
VS	5,108	1,724	1,472	71.2	39.1
TKN	780	195	60	92.3	58.0
TN	780	481	435	44.2	24.7
NH_3-N	510	120	2.5	99.5	99.5
NO_3-N	0	37	195		
NO_2-N	0	249	180		
pH	8.1	6.8	7.9		

Table 19. Performance of the Two-Stage SBR-CMBR System for 20,000 mg/L Influent COD

Parameters	Influent (mg/L)	Stage I: SBR (1-Day HRT) Liquid Effluent (mg/L)	Stage II: CMBR (1-Day HRT) Liquid Effluent (mg/L)	E_I (%)	E_t (%)
COD	20,000	4,300	2,676	86.6	37.0
SCOD	6,660	3,197	2,020	69.7	69.7
TS	12,442	4,367	3,432	72.4	21.8
VS	10,104	3,142	2,152	78.7	27.0
TKN	1,140	540	180	84.2	46.1
TN	1,140	573	504	55.8	23.2
NH_3-N	540	310	3.0	99.4	99.4
NO_3-N	0	20	190		
NO_2-N	0	13	134		
pH	8.0	8.7	7.8		

It is obvious that adding the second-stage reactor in the treatment system can help achieve near-complete ammonia conversion. Both first-stage and second-stage SBRs were operated at one-day HRT with the system HRT being two days. The liquid effluent from the second-stage SBR contained little residual ammonia when the SBR was used either to treat the partially-nitrified liquid effluent from the first-stage SBR at 1-day HRT and 10,000 mg/L influent COD or to treat the not-nitrified liquid effluent from the first-stage SBR at 1-day HRT and 20,000 mg/L influent COD.

The effluent quality and removal efficiencies of COD and solids from the two-stage system at 2-day HRT were comparable to those from the single-stage SBR at 3-day HRT. This suggests that based on the HRT, the two-stage system would require 1/3 less reactor volume than the single-stage system and therefore appears to have more favorable economics. In addition, the two-stage system allows more complete nitrogen conversion in the wastewater. Therefore, if nitrification is desired, the two-stage system configuration is recommended over the single-stage system.

The treated liquid effluent can be stored separately and used as nitrate-rich irrigation water. Since there is little ammonia contained in the liquid effluent, ammonia emission from the storage will be minimal. If nitrogen removal is desired, denitrification may be induced in the storage to convert nitrite and nitrate into nitrogen gas. Future study is needed to study the stability of nitrite and nitrate in the effluent during storage and to determine the best conditions for denitrification.

2.5.3.2 Field Research Results

The total solids content of dairy wastewater entering the SBR system varied in the range of 1 to 3.5 percent. Figure 23 and Figure 24 show the performance of single-stage SBR in terms of influent and effluent characteristics and removals of TS and VS. Solids concentrations (TS and VS) in the effluent of the SBR was consistently low, 0.4 to 0.6 percent TS.

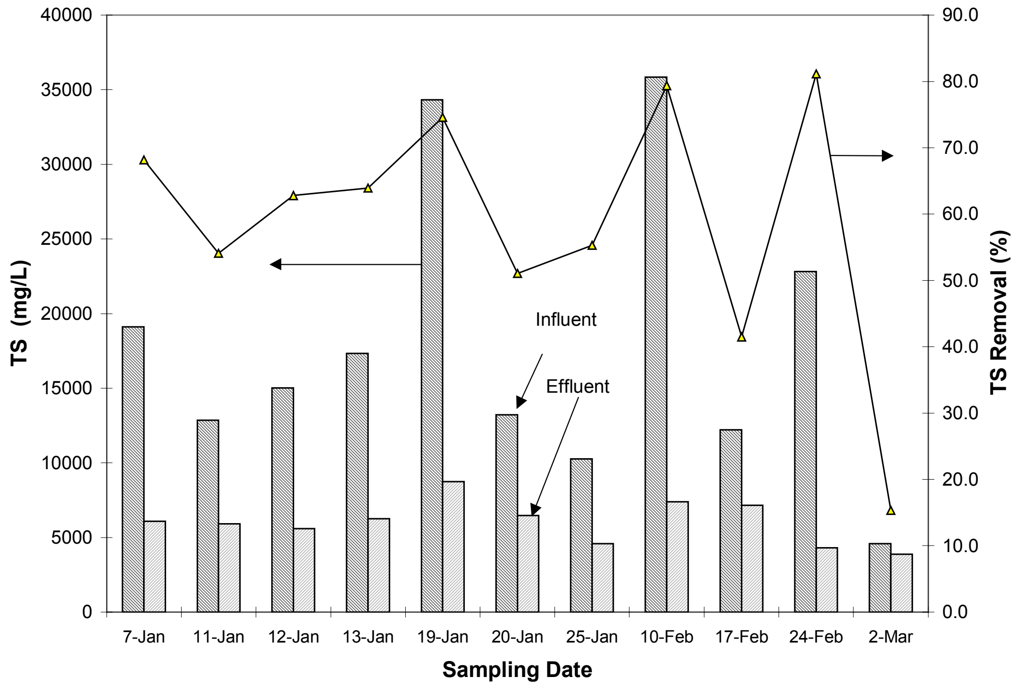


Figure 23. Performance of the Single-Stage SBR in Removing Total Solids From Dairy Wastewater

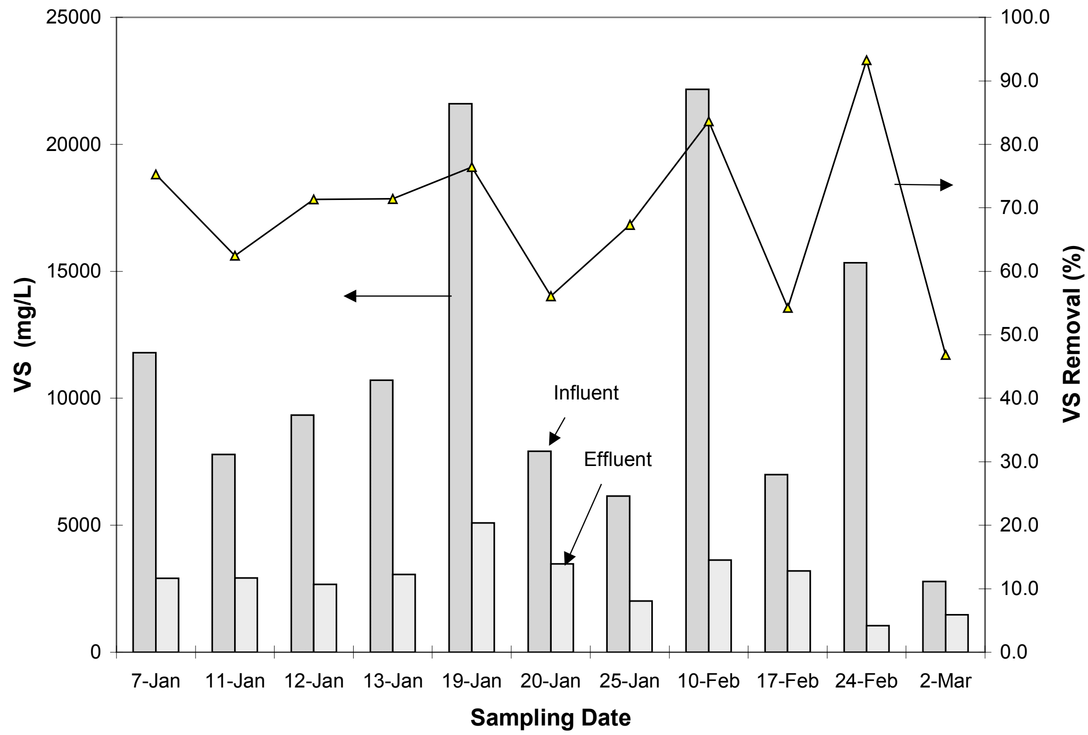


Figure 24. Performance of the Single-Stage SBR System Removing Volatile Solids

The removals of TS and VS were up to 80 percent and 90 percent, respectively. Therefore, the single-stage SBR treatment with a 3-day hydraulic retention time was effective in removing solids from the dairy wastewater. The nitrogen removal, however, was not high. The TKN removal of 28 to 52 percent was achieved. The nitrogen removal was largely due to solids settling rather than biological conversion.

There was 7 to 26 percent ammonia loss during the treatment. This might be due to ammonia volatilization and simultaneous nitrification and denitrification. The latter cause is considered to be minor because little nitrate or nitrite was detected in the SBR.

Adding the second-stage SBR into the treatment system increased nitrogen conversion significantly. Figure 25 and Figure 26 show the reduction of 98 to 100 percent for NH3-N and 83 to 91 percent for TKN achieved. The pilot-scale testing of the SBR system confirmed the findings of the laboratory study in that the two-stage SBR system is more effective and energy efficient than single-stage SBR in removing nitrogen from the dairy wastewater.

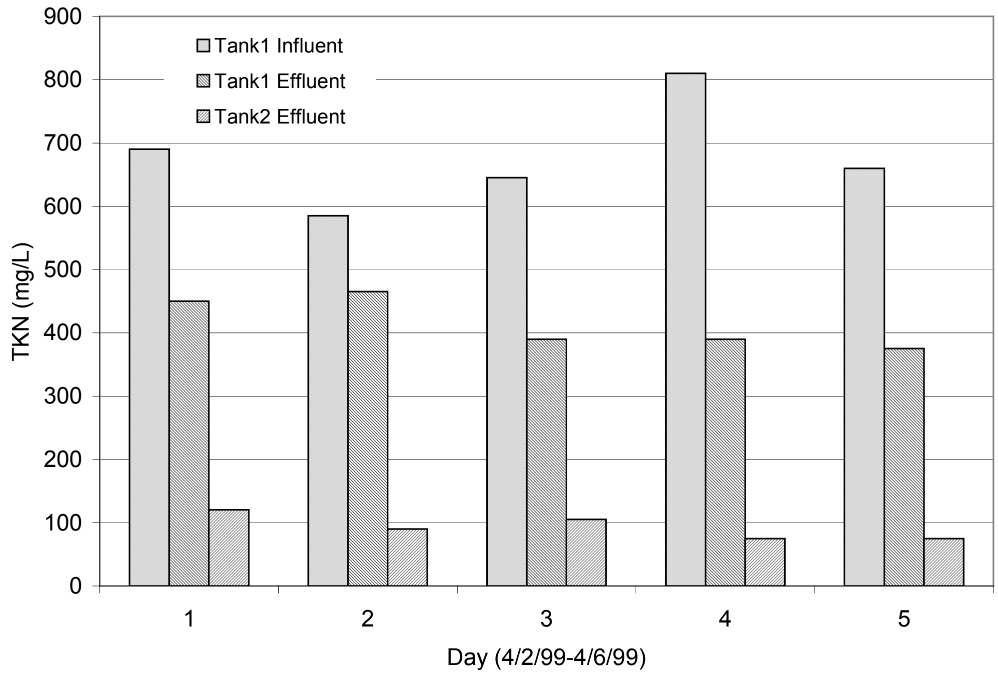


Figure 25. The TKN in the Influent and Effluent of the Two-Stage SBR System

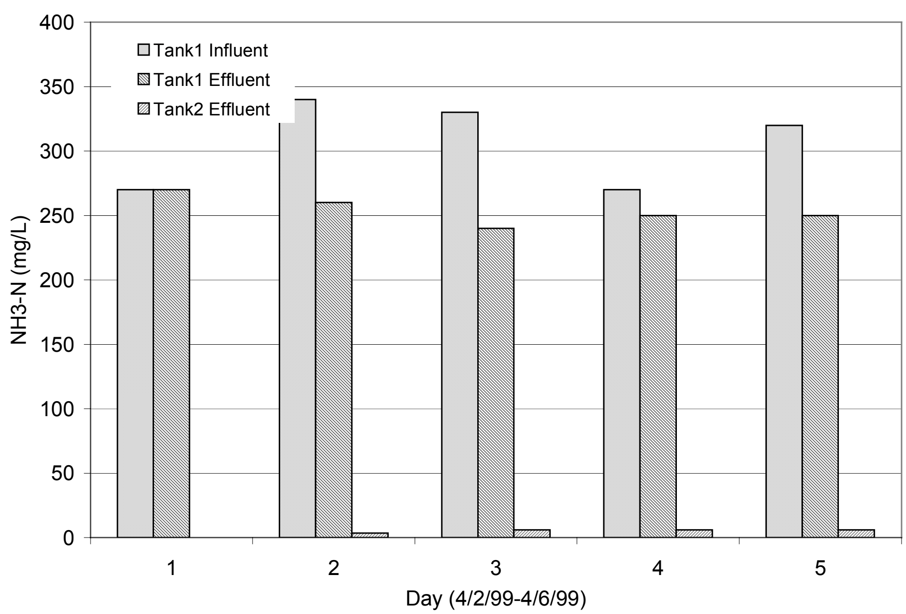


Figure 26. Ammonia Nitrate in the Influent and Effluent of the Two-Stage SBR System

2.5.4 Conclusions and Recommendations

The sequencing batch reactor was found to be an effective biological reactor for treating dairy wastewater. The hydraulic retention time, solids retention time and the contents of organic solids and nitrogen in the influent are important parameters that could affect the treatment efficiencies of the SBR.

A two-stage SBR treatment system is recommended over the single-stage system if nutrification is desired. The two-stage system was capable of achieving near-complete conversion of ammonia to nitrite and nitrate in the dairy wastewater. The system HRT of the two-stage system was 1/3rd shorter than the HRT of the single-stage system for treating the wastewater of 20,000 mg/L COD. These results indicate that the two-stage system has more favorable economics and effective nutrification performance.

2.6 Irrigation Scheduling

California's agricultural community is the single greatest user of water in the state. As the state's population and major urban centers have each grown, water use by the agricultural community is coming under increasing scrutiny and criticism as being wasteful. Moreover, the energy demands imposed by irrigation requirements represent the single most significant component of the energy budget for most growers of row and orchard crops.

Modern farming practices include three primary methods to scientifically schedule crop irrigation. These include:

- Atmospheric based (water budget using estimates of evapotranspiration)
- Soil based (using measurements of soil moisture content)
- Plant based (based upon direct or indirect measurements of plant water content).

Recent advancements in electronic measurement techniques, data collection, and analysis suggest that the scientific methods described above may be applied with greater precision by the use of automated data monitoring and analysis procedures. The project activity associated with this task evaluated the effectiveness of each irrigation scheduling method using electronically collected indicator proxies to define irrigation regimes.

Improved irrigation efficiency will lead to reduced water usage and energy expenditure by California's fruit and nut orchard operators, thereby conserving both water use and energy expenditures. This task will demonstrate by a field experiment where the above methods of irrigation scheduling can be compared and evaluated.

2.6.1 Project Objective

The objective of this project was to investigate innovative techniques for improving irrigation efficiency for fruit and nut orchard crops. Growers currently rely mostly on historical irrigation scheduling information; primarily management practices they have deemed successful in the past. Only a few growers utilize real time-based monitoring techniques. The relatively slow adoption of improved techniques is principally due to cheap water and energy; these factors are not strong motivation for change. Demonstrating that improved techniques can save growers money and produce top yields of high quality fruit would make adoption of the improved techniques much more likely.

2.6.2 Project Approach

There are three primary methods for scientific irrigation scheduling: atmospheric based (water budget using estimates of evapotranspiration), soil based (taking measurements of water content or water status), and plant based (directly or indirectly measuring plant water status). Recent advancements in electronic data collection and transfer suggest that automated monitoring of pertinent parameters can be used to improve irrigation efficiency in California's fruit and nut orchards, thus saving water and energy.

This work took place on about a one-acre site at Edison AgTAC in Tulare, California. In early 1998, 20 plots, each three trees wide and five trees long, were planted with peach "September Snow," a white flesh variety. This allows for four replications of the five irrigation regimes. For the four replications of the four irrigation regimes, the vines (variety “Shiraz”) were planted in 16 plots, again with a 3 x 5 configuration. The interior three trees/vines were used as the measurement plants. The irrigation system originally consisted of micro-sprinklers on the peach trees and drip irrigation of the vines. It became apparent in the first season that the application rate (6 gal/hr) of the micro-sprinklers (one per tree) was resulting in ponded and runoff water. In early 1999, the micro-sprinklers were changed to drip (two emitters at 0.5 gal/tree). The irrigation systems for each irrigation treatment are operated independently.

Five irrigation methodologies were used in the trees.

- **Water Budget** – The automated CIMIS weather station in Visalia was used to collect hourly data on temperature, humidity, wind, solar radiation, and rainfall. The data was processed to estimate reference crop water use. Peach crop coefficients from planting to orchard maturity that have been previously determined were used to estimate orchard water use. These data were determined weekly and are used to set the irrigation system operating times for the following week.
- **Soil** – EnviroScan probes that automatically collect soil water contents at the 20, 50, 90, and 150 cm depths were installed in this treatment. The goal was to keep soil water at a optimal, constant level throughout the season. It was determined that this level was 400 mm based on readings taken at the beginning of the season when the profile was full. The irrigation was managed to keep soil water at this level.
- **Plant Water Status** – After having placed a foil covered, polyethylene bag on interior leaves for 2 hours, a pressure chamber was used to determine stem water potential (SWP) at about 1 p.m. Readings were taken twice per week. Threshold values indicating well-watered status were established and irrigation was performed accordingly.
- **Trunk Diameter Fluctuations** – Linear variable displacement transducers (LVDTs) were used to continuously monitor trunk diameter. Maximum daily trunk shrinkage (MDS), the difference between the maximum trunk size that occurs in the early morning hours and the minimum trunk size that occurs in the late afternoon, was the parameter used to indicate stress. The irrigation system is adjusted to maintain minimal changes in MDS in this treatment.
- **Control** – To evaluate the performance of each of these irrigation scheduling treatments, it was necessary to have a fully irrigated control for comparison. This treatment was irrigated at 150 percent of the water budget irrigation. This proved to be excessive and in July, was reduced to 125 percent.

Fruit diameter (four fruit/monitored tree) was observed once per week.

2.6.3 Project Outcome

Applied water varied with the different irrigation regimes (Figure 27). Through mid-July, most water had been applied with the control (15.7 inches) and the least with the EnviroScan (9.8 inches).

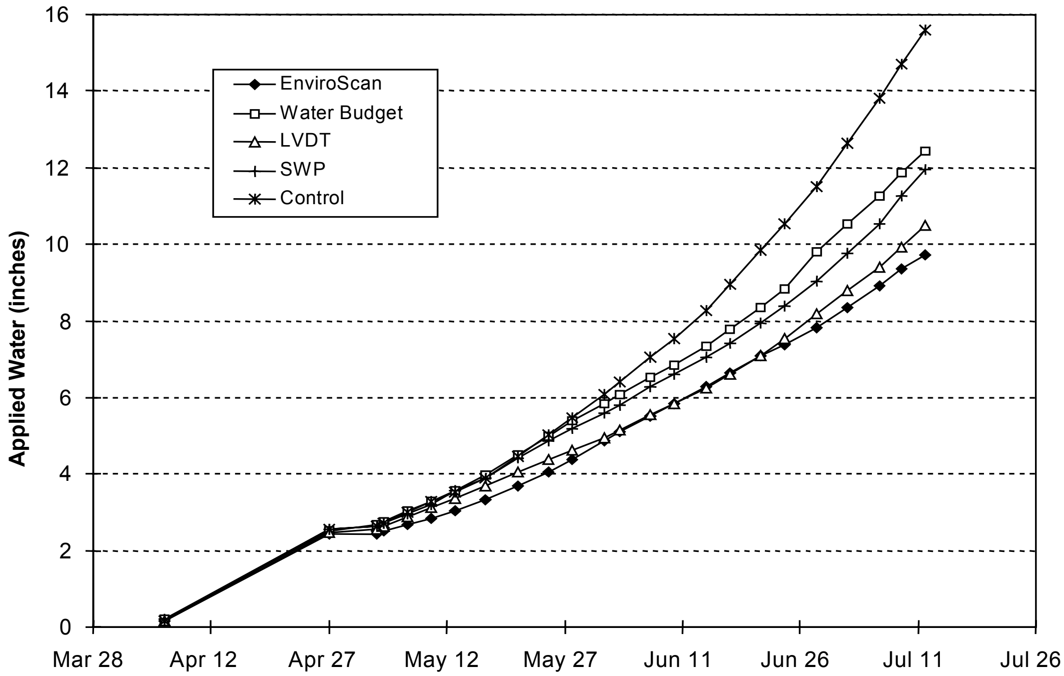


Figure 27. Applied Water for Each Irrigation Treatment

Stem water potential was nearly identical until mid-June when control values increased; they became less negative (Figure 28). This suggests that all the other regimes were stressed during that period. Others have found that excessive irrigation reduced stress even though no additional tree water use took place. This is a disturbing phenomenon for those interested in minimizing deep percolation.

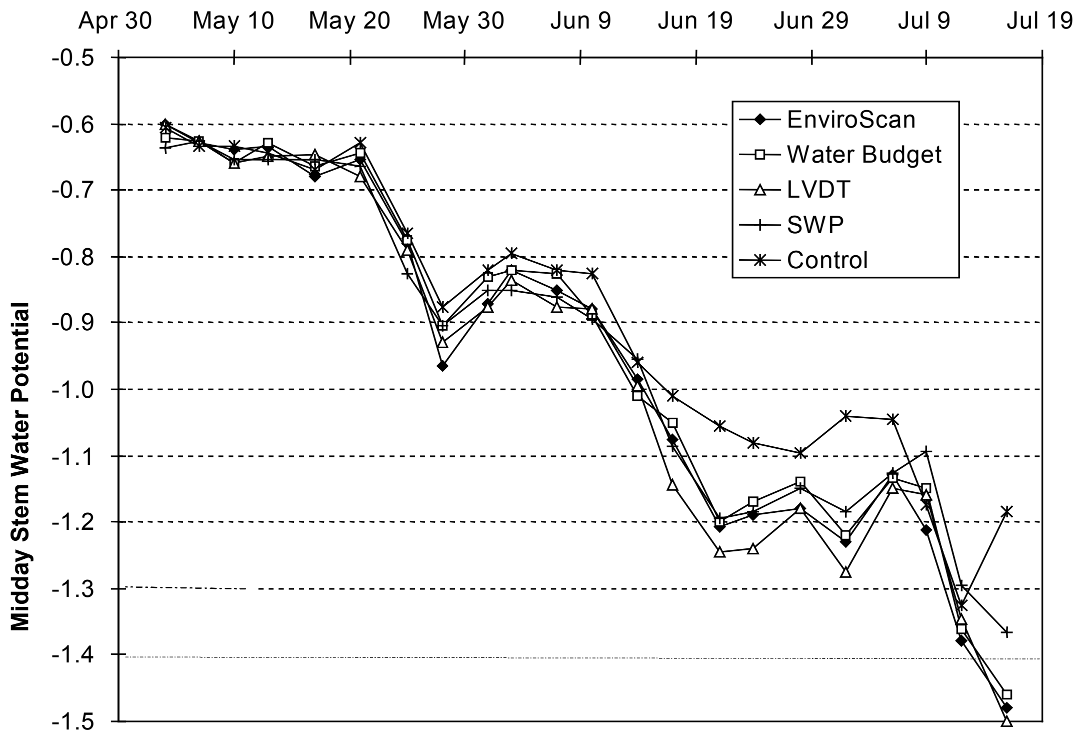


Figure 28. Midday Stem Water Potential with Time

Trunk diameter fluctuations were as expected (Figure 29). MDS was calculated daily from this LVDT data.

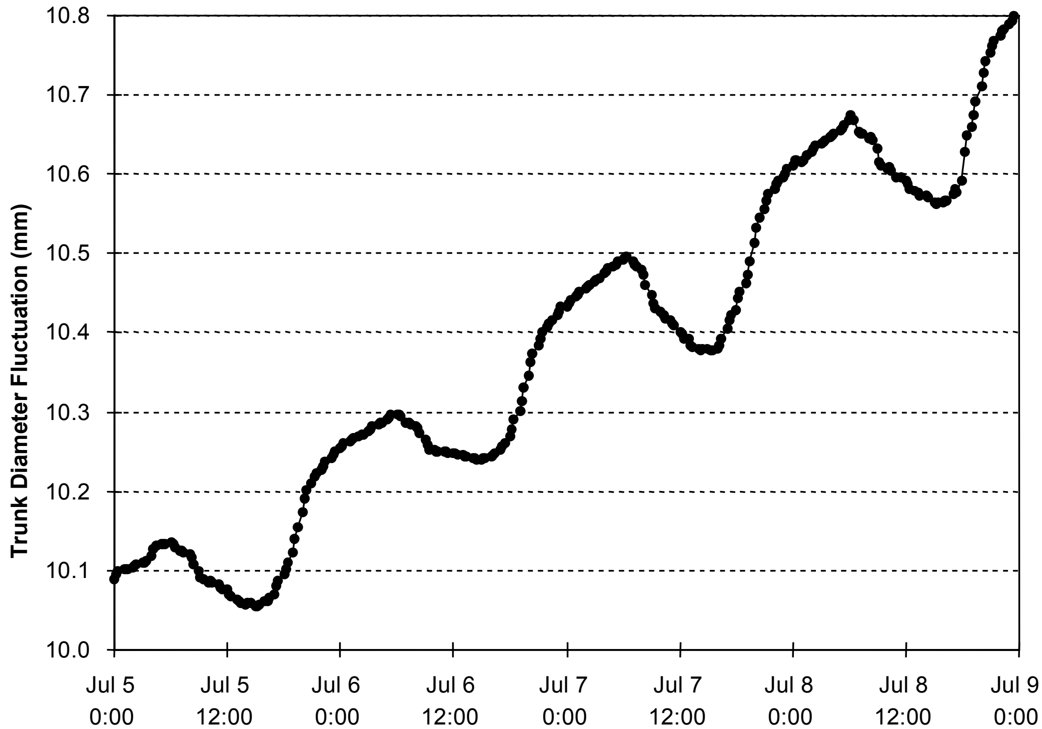


Figure 29. Example of LVDT Data
Midnight is 0:00 and Noon is 12:00

Figure 30 shows maximum daily trunk shrinkage. There was little difference between irrigation treatments until mid-June; the same time the SWP differences occurred. At that time, control values were less than the other irrigation regimes, again indicative of stress.

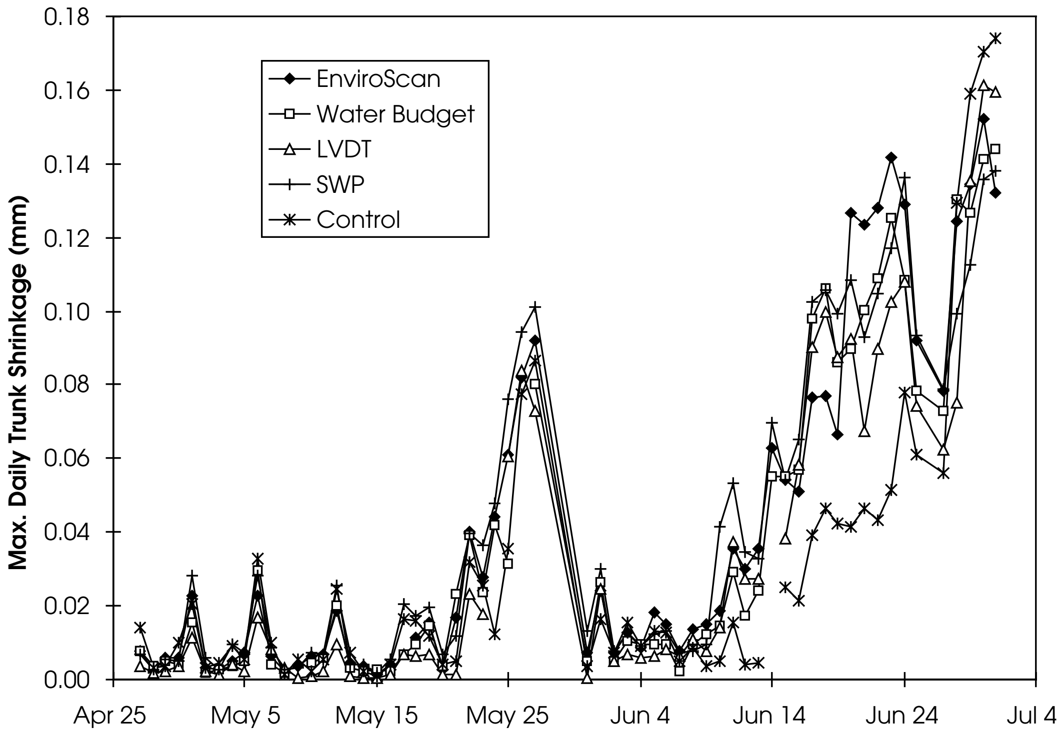


Figure 30. Maximum Daily Trunk Shrinkage (MDS) With Time

Maximum daily trunk shrinkage (determined for the LVDT plot) and SWP for the same trees were highly correlated with an R2 of 0.794 (Figure 31). This is strong evidence that LVDTs can be used as a surrogate for SWP, providing, for the first time, a method to electronically monitor plant water status.

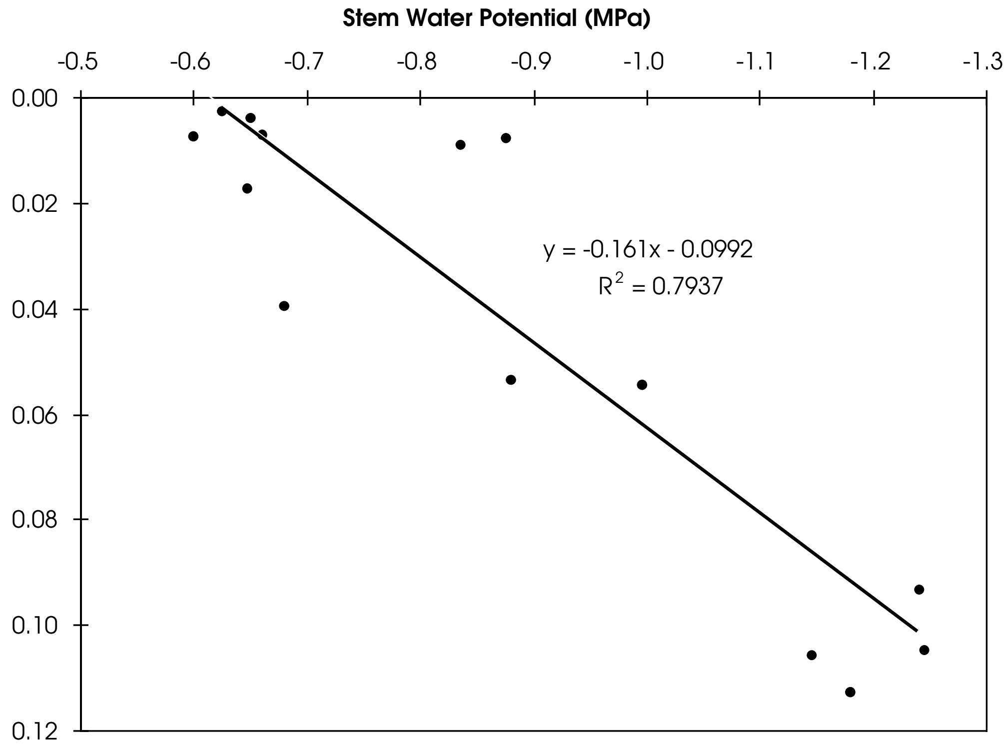


Figure 31. Relationship between MDS and SWP Measured in LVDT Plots

One of the barriers to adoption of SWP measurements in production agriculture is the process of having to go to the field a few hours before the measurement to bag the leaves. It was theorized that the water potential of shaded, interior leaves might correlate well with SWP. Shaded leaves were sampled in both the water budget and control treatments. Figure 32 shows the correlation between shaded leaf water potential and SWP for both treatments. The strong correlation (R2 of 0.969) indicates that measuring shaded leaf water potential can be used as a surrogate for SWP, removing one of the barriers to adoption.

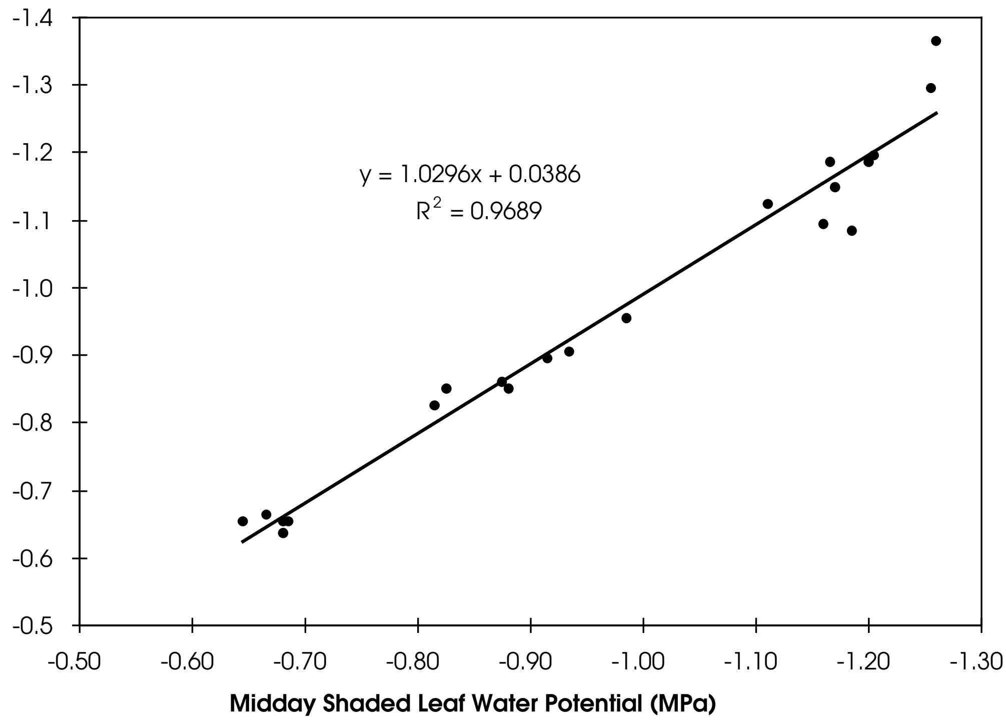


Figure 32. Relationship between SWP and Shaded Leaf Water Potential
Measured in Water Budget and Control Plots

Through mid-July, there was little difference in fruit size between the irrigation regimes (Figure 33). This would be the key yield component to evaluate the impact of the different scheduling techniques on production.

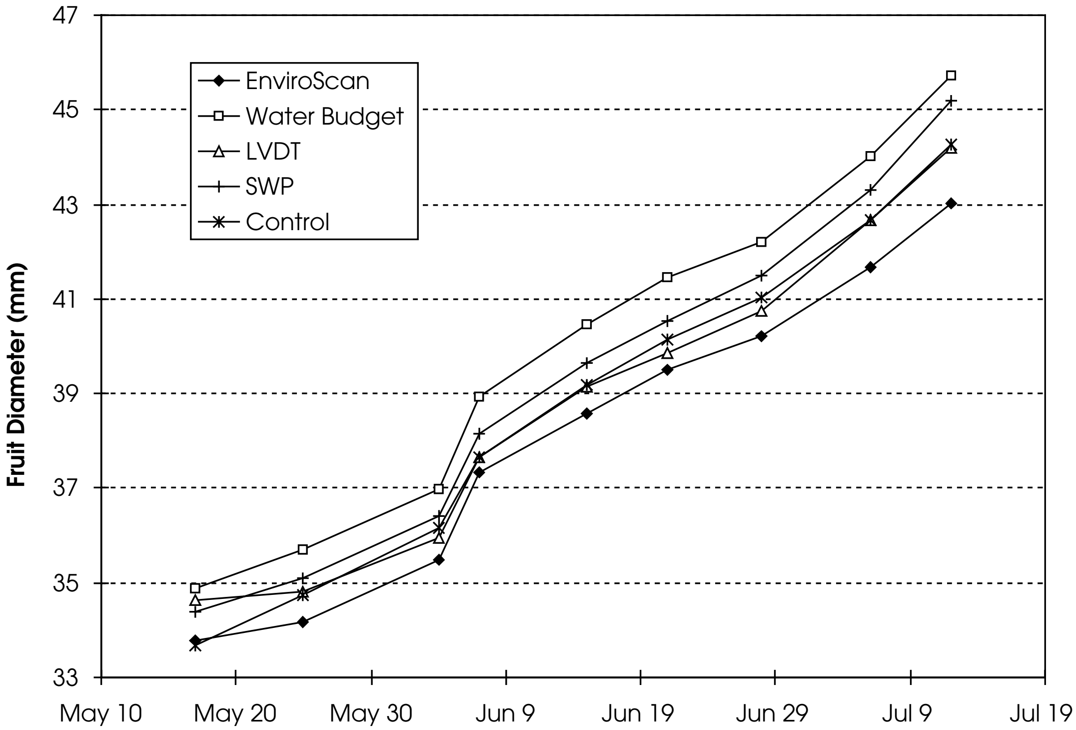


Figure 33. Fruit Growth with Time

2.6.3.1 Conclusions and Recommendations

The objective of this project was to investigate advanced techniques to improve irrigation efficiency for fruit and nut orchard crops.

The project was designed with two parts. The first involved the comparison of advanced irrigation scheduling methods. The second part was to use plant, soil, and atmospheric sensors as indicators for the management of regulated deficit irrigation (RDI). Each part was to last for 2 years. This time constraint required that the trees and vines achieve maximum possible growth during the first two seasons to be at full vegetative cover at the start of year 3.

Due to a late winter preceding the first year (1998), early growth was slow, especially in the vines. We were successful in encouraging fast vegetative growth in the trees in the remainder of the first season to achieve about 25 percent cover at the start of year 2. This allowed us to begin irrigation treatments in 1999 in the trees. The vines had to be pruned back to leave only two buds at the end of 1998 due to poor and variable growth the first year. Thus, 1999 was used to train the vines up the stakes and onto the wires to achieve the cordons necessary to being the irrigation treatments in 2000.

The goal of the first phase of this project was to evaluate the impact of different irrigation scheduling techniques on tree growth and fruit yield and quality. Only 3 months of data, however, has been collected to date and harvest results will not be available until September.

It is recommended that field trials be continued to their scheduled conclusion at which time more meaningful research results should be available.

3.0 Conclusions and Recommendations

This program was funded to analyze and demonstrate numerous energy efficient and environmentally friendly agriculture-related technologies. Program findings will help California farmers enhance competitiveness; improve productivity; and reduce the use of toxic chemicals, energy consumption, and water usage.

The program consisted of six projects, whose objectives were to demonstrate:

- The effectiveness of ozone as a preplant soil fumigant to destroy a variety of soil-borne microorganisms
- The effectiveness of ozone as a disinfectant and fungicide in aqueous solutions used for fruit storage and packaging operations, and ozone treatment for discharge water quality purposes
- The effectiveness of ozone as a post-harvest fumigant to control insect infestation of fresh and dried fruits and vegetables
- The practicality of using low temperature control and ventilation strategies and/or plastic bin liners as substitutes for methyl bromide in the insect control of stored prunes
- The functionality of an innovative biological treatment device to manage the disposal of liquefied animal wastes
- New and advanced techniques for improving irrigation efficiency for fruit and nut orchard crops.

Major conclusions and recommendations for each of these projects are provided in the following. A detailed discussion for each task is included in Section 2.0 of this document.

Ozone in Soil Fumigation

The results of extensive field trials generally demonstrate the broad effectiveness of ozone treatment of soil in increasing plant yield and reducing the detrimental effects of soil pathogens. These tests were performed on a variety of crops and soil types under a range of climatic conditions. In every trial except the peach trial, substantial improvements in crop yield or plant vigor resulted from the ozone preplant application compared to untreated controls. In many cases where alternative fumigants were also tested, the best ozone treatment often exceeded one or more of the conventional fumigant treatments.

The study concludes that soil treatment with ozone results in decreased soil pathogen pressures and increased nutrient availability. Together, these benefits promote increased plant growth and yield without any detrimental environmental effects. Additional work is necessary, however, to be able to accurately predict the specific growth response achieved by ozonation in different crops, soil types, and climatic conditions.

Modest levels of phytotoxicity were noted in the form of lower leaf burn in a number of plants in several plots upon midseason ozone applications in the several trials. In addition, the yield of tomato plots receiving midseason ozone applications at one site showed a slightly lower yield than those plots not receiving midseason ozonation dosages. In contrast, strawberry plots which received midseason ozonation applications showed substantially increased growth compared to plots which received only a preplant treatment. Further work is obviously needed to properly define the dosage levels that yield the maximum growth response without phytotoxicity.

The effects of mixing carbon dioxide with the ozone gas when injected as preplant treatment were mixed. In the case of some tomato and carrot trials, and the sugar beet trials in Tulare, coextensive use of carbon dioxide resulted in increased yield. The opposite effect was seen in the carrot and sugar beet trials in the nematode-laden soils in Irvine. Further field trials are necessary to properly predict the effects of such treatments in future applications.

Ozone as an Aqueous Disinfectant

While it is premature to declare conclusions on many aspects of this project, ozone clearly has shown promise as a sanitizer to minimize chemical and microbial contamination of process water that contacts fruit during post-harvest handling. Sanitation of fruit surfaces is achievable, but requires lengthy contact times compared to other sanitizers. In addition, a high ozone concentration is required. Ozone acted suitably as a replacement for hypochlorite for the control of gray mold, but tests indicated some loss in efficacy.

Ozone is compatible with bicarbonate salts, a simple and effective treatment for many post-harvest diseases. Ozone could increase the life of bicarbonate solutions, used as a simple, safe and effective treatment for many post-harvest diseases. The use of ozone reduces biological oxygen demand and clarifies the solution, and it kills nuisance microbes that contaminate repeatedly-used bicarbonate solutions.

Ozone could also have a role in reducing fungicide residues in discharge water. More research to assess the benefits of ozone in water treatment on other commodities, such as peaches, plums, and nectarines, should be conducted.

Some limitations in the use of ozone for post-harvest fruit treatment were discovered. Tests exposing the immobile stage of California Red scale on lemons to ozone in a water bath showed that scale could not be eliminated on the citrus even after a 20-minute exposure at the highest level of ozone concentration possible.

Ozone as a Gas Fumigant

Results of exposing larvae of the Indianmeal moth and diapausing coddling moths to ozone gas showed that from four to 6 hours were needed to kill the larvae at concentrations of 300 to 500 ppm of ozone. Other insects were not tested because of time constraints, but these exposures and other tests are continuing to determine efficacy of ozone in this application.

Because testing of insects was not complete, the second phase of the project was not initiated. As a clear picture of the efficacy to insects emerges, testing of several commodities for the effects of ozone on the commodity will be completed.

While ozone was shown to be toxic to insect larvae, the concentrations required were high and the exposure times long. Ozone at these levels, 300 to 500 ppm, is corrosive due to its oxidizing characteristics. Chambers in which to conduct fumigations would require materials able to withstand the corrosive action of continual exposure to the high ozone concentrations.

Alternative Fruit Storage Methods

Initial activity on this project revealed that the cost of the proposed controlled ventilation/evaporative cooling system was prohibitively expensive when compared to available storage methods. Continued effort on this project was halted with this determination.

Field and lab tests indicated that storage in plastic-film bin liners protected fruit quality and provided a low-cost method of protecting fruit from reinfestation. Tests demonstrated, however, that the bin liners were not consistently effective in excluding Indianmeal moth. Exclusion efficacy may be improved by better bag sealing methods. Future research should be done to improve bag storage methods.

Dairy Wastewater Management

The technology identified for demonstration, the sequencing batch reactor (SBR), was found to be an effective biological reactor for treating dairy wastewater. Both a single-stage and a two-stage SBR were demonstrated and evaluated. The two-stage SBR treatment system is recommended over the single-stage system if nitrification is desired. The two-stage system was capable of achieving near-complete conversion of ammonia to nitrite and nitrate in the dairy wastewater.

Irrigation Scheduling

While conclusive results are not available after only 4 months of monitoring, and prior to any fruit harvests, following key observations were made as a result of this project.

It was found that the linear variable displacement transducers provided an accurate method to electronically monitor plant water status; the first step in an automated irrigation scheduling system responsive to specific plant needs. In addition, strong correlation between the use of shaded, interior leaves for water measurement and the previous method of water measurement, the labor-intensive leaf bagging, provided evidence of the accuracy of measuring stem water potential through the use of shaded leaves. The availability of a less labor intensive process removes one of the barriers of adoption of stem water potential measurement in effective irrigation.

It is recommended that field trials continue to their scheduled conclusion to ensure meaningful research results from this project.

Notes

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